# FABRICATION OF β- CYCLODEXTRIN LOADED GLIMEPIRIDE NANOPARTICLES AND ITS EVALUATION

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**MASTER OF PHARMACY**

**IN**

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**THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY,**

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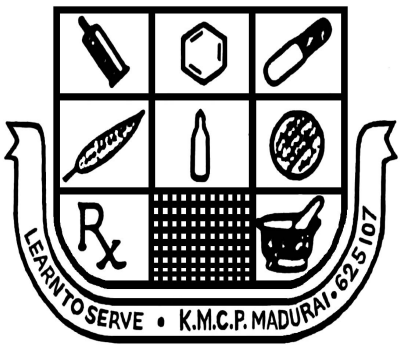


**DEPARTMENT OF PHARMACEUTICS**

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# CERTIFICATE

This is to certify that the dissertation entitled **"FABRICATION OF β-CYCLODEXTRIN LOADED GLIMEPIRIDE NANOPARTICLES AND ITS EVALUATION"** submitted by **Ms. JACQULIN (reg. No: 261210103)** in partial fulfilment of the degree of Master of Pharmacy in Pharmaceutics under the Tamilnadu Dr.M.G.R Medical University, Chennai, done at **K.M. COLLEGE OF PHARMACY,** MADURAI-625107, is a bonafide work carried out by her under my guidance and supervision during the academic year APRIL-2014. The dissertation partially or fully has not been submitted for any other degree or diploma of this university or other universities.

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## CONTENTS

## LIST OF SYMBOLS AND ABBREVIATIONS

| ABBREVIATIONS | FULL FORM |
|---|---|
| nm | Nanometre |
| o/w | Oil in water |
| PVA | Poly vinyl alcohol |
| E.E | Entrapment efficiency |
| DM | Diabetes mellitus |
| % | percentage |
| β- CD | Beta cyclodextrin |
| $^0$C | Degree Celsius |
| Hrs. | hours |
| GIT | Gastro intestinal tract |
| UV | Ultra violet |
| FT-IR | Fourier transform infrared |
| mg | Milligram(s) |
| g/cm | Gram per centimetre |
| µm | micrometre |
| PEG | Poly ethylene glycol |
| µg/ml | Microgram per millilitre |
| λ | wavelength |
| gm | Gram(s) |

**NANOPARTICLES:**

To obtain a desirable therapeutic response, the correct amount of the drug should be transported and delivered to the site of action. The distribution to other tissues therefore seems to be a potential cause for toxicity. Targeted drug delivery system involves a number of essential bio ligands for bio signalling and physiological cell need. They are operated through bio ports which are referred as receptors. The ligand-receptor interactions are highly stereo specific. Therefore ligands or receptors could be exploited for cell specific drug delivery.

**Table 1: CARRIER SYSTEMS USED FOR TARGETED DRUG DELIVERY:**

| S.No | Category | Example |
|---|---|---|
| 1 | Colloidal carriers | a) Vesicular systems Niosomes,liposomes,virosomes, pharmacosomes, immune liposomes<br>b) Micro particulate systems Nanoparticles,microparticles,nanocapsules, magnetic microspheres, albumin microspheres. |
| 2 | Cellular carriers | Resealed erythrocytes, antibodies, serum albumin, platelets and leukocytes. |
| 3 | Supra molecular delivery systems | Micelles, mixed micelles, reverse micelles, polymeric micelles, lipoproteins, liquid crystals, synthetic LDL. |
| 4 | Polymer based systems | Mucoadhesive, biodegradable, signal sensitive, bio erodible. |
| 5 | Macromolecular carriers | a) Proteins, glycoprotein, neo glycoproteins, artificial viral envelopes.<br>b) Glycosylated water soluble polymers (poly-lysine).<br>c) Mabs, antibody enzyme complex.<br>d) Toxins, immunotoxin and rCD4 toxin conjugates.<br>e) Lectins and polysaccharides. |

## COLLOIDAL DRUG CARRIER SYSTEM:

The colloidal carriers based on biodegradable and biocompatible polymeric systems have largely influenced the controlled and targeted drug delivery concepts. Nanoparticles are composed of synthetic or semi synthetic polymers. Nanotechnology is the study of nanoparticles, nanosuspension, nanoemulsion, etc., Nanoparticle is a collective name for nanospheres and nanocapsules. Drugs absorbed at the surface, entrapped in the particle or dissolved in it are called Nanospheres. The active substance dissolved in the inner core, or absorbed at the surface is called nanocapsules.

Nanosuspension consists of essentially pure drug and minimal quantities of surface stabilizing agents; it provides sustained release at a level below the maximum tolerated dose yet at its therapeutically effective concentration. They are mainly used for parentral drug delivery.

**Table 2: VARIOUS CARRIER BASED DOSAGE FORMS:**

| S.NO | CARRIER SYSTEM | SIZE RANGE | FEATURES | METHOD OF PREPARATION |
|---|---|---|---|---|
| 1 | Nanoparticles | 10-1000nm | Submicron–sized Colloidal systems,biodegradable (or) not | Emulsion solvent evaporation method |
| 2 | Solid lipid nanoparticle | 50-1000nm | Submicron colloidal carriers containing solid hydrophobic core having a monolayer of phospholipids coating | High-pressure homogenization Micro emulsion formation precipitation as lipid nanopellets |
| 3 | Polymeric nanoparticles | 10-1000 nm | Sub-nanosized colloidal structure composed of synthetic or semi -synthetic polymers | Nano precipitation method |
| 4 | Lipid emulsion | Lipid globule | Multi component fluid made of water. A | o/w w/o |

| | | | | |
|---|---|---|---|---|
| | | 1 -100nm | hydrophobic liquid, or several surfactants resulting in a stable system | w/o/w |
| 5 | Liposomes | 25-100nm | Microscopic vesicles composed of one or more concentric lipid bilayer, separated by water or aqueous buffer compartments | Nano precipitation method |
| 6 | Lipospheres | 0.2-100nm | Water dispersible solid micro particles composed of solid hydrophobic fat core stabilized by a mono layers of phospholipids molecules embedded in a micro particles surface | Melt method Co-solvent method, Pre incorporation into lipophilic carrier |
| 7 | Lipid microtubles/ Micro cylinders | 1nm | Self-organizing system in which surfactants crystallize into tightly packed bilayers that spontaneously form cylinders | Self-emulsification |
| 8 | Ceramic nanoparticles | 50nm | Made up of inorganic (ceramic) compounds such as silica, titania and alumina | Emulsion solvent evaporation method |
| 9 | Functionalized nanocarriers/quantum dots | | Combination of functionalities of biomolecules and nano biologically derived molecular species | |
| 10 | Nanotubes and nanowires | | Self-assembling sheet | Chemical |

| | | | | |
|---|---|---|---|---|
| | | | of atoms arranged in the form of tubes and thread like structures of nanoscale range | evaporation dispersion |
| 11 | Multi composite ultra thin capsules | 50nm few micron | Molecular assemblies of tailored architecture having layer-by-layer adsorption of oppositely charged macromolecules on to colloidal particles | Langmuir-Blodgett technique and chemisorptions from solution |
| 12 | Ethosomes | | Nanoinvasive delivery carriers that enable drugs to reach the deep skin and systemic circulation | Ethanol injection-sonication method |
| 13 | Niosomes | 12-16 micron | Non-ionic surfactant vesicles are bi-layered structures | Ether injection method. |
| 14 | Collidosomes | | Solid microcapsules which are hollow, elastic shells | Self-assembly of colloidal particles at the interface of emulsion droplets |
| 15 | Pharmacosomes | | Pure drug vesicles formed by the amphiphilic drugs | |
| 16 | Dendrimers | | Macromolecular compounds that consists of a series of branches around an inner core | Polymerization |

**POLYMERIC NANOPARTCLES:** [1, 2]

These are colloidal particles ranging from 10-1000 nm which consist of macromolecular materials. This can be used therapeutically, e.g. as adjuvant in vaccines, drug carriers in which the active principle (drug or biological active material) is dissolved, entrapped or encapsulated. The active principle is adsorbed or attached. Polymeric nanoparticles are composed of biodegradable or bio stable polymers and copolymers. The active agents can be;

I. Entrapped or encapsulated within the particles

II. Physically adsorbed on surface (or)

III. Chemically linked to the surface of the nanoparticles.

**POLYMERS USED IN THE PREPARATION OF POLYMERIC NANOPARTICLES:**

Polymers used in manufacturing of polymeric nanoparticles are of two types;

1. Natural hydrophilic polymers (proteins and polysaccharides)
2. Synthetic hydrophobic polymers (poly lactic acid and PLGA)

**Natural hydrophilic polymers:**

Gelatin, albumin, vicilin or legumin as well as polysaccharides like agarose or alginates have been studied and characterized. These macromolecules are used due to their intrinsic biodegradability and biocompatibility.

**Synthetic hydrophobic polymers:**

Polymers that are used for microsphere preparation are used for the preparation of nanoparticle. Most of them are hydrophobic in nature. The polymers are either synthesized or pre-polymerized during nanoparticles preparation.

**PREPARATION TECHNIQUES OF NANOPARTICLES:** [3, 4, 5.]

The preparation of nanoparticles depends on the physicochemical properties of the polymer and the drug to be loaded. Two types of system with different inner structures are apparently possible. They are:

1. A matrix type system consisting of an entanglement of oligomer or polymer units. (nanoparticles or nanospheres)

2. A reservoir type of system comprised of an oily core surrounded by an embryonic polymeric shell (nanocapsules)

   The drug can be either entrapped within the reservoir or matrix or adsorbed on the surface of these particulate systems. They are classified as;

Amphiphilic macromolecule cross-linking

- Heat cross linking
- Chemical cross-linking

Polymerization based methods

- Emulsion (micellar) polymerization
- Interfacial condensation polymerization
- Interfacial complexation
- Polymerization of monomers *insitu*
- Dispersion polymerization

Polymer precipitation methods

- Solvent displacement (nanoprecipitation)
- Solvent extraction or evaporation
- Salting out

**FORMULATION OF NANOPARTICLE USING POLYMER PRECIPITATION METHOD: [6.]**

The hydrophobic polymer and hydrophobic drug is dissolved in an organic solvent. It is then dispersed in a continuous aqueous phase in which the polymer is insoluble. Precipitation of the polymer produces nanoparticle with the drug loaded in it.

Polymer precipitation is done by increasing the solubility of the organic solvent in the external medium by adding alcohol.

**Solvent extraction method:**

The preparation of nanoparticles begins with the formation of conventional O/W emulsion between partially water miscible solvent containing the polymer and the drug, and an aqueous phase containing stabilizer. The removal of the solvent (solvent evaporation method) or addition of water to diffuse the solvent to the external phase (emulsification diffusion method) is the two types of solvent extraction method.

**Double emulsion solvent evaporation method:**

Emulsion solvent evaporation method is modified and double emulsion of W/O/W type has been used. Nanoparticles are formed by the evaporation of organic solvents. This is then recovered by centrifugation, washed repeatedly with buffer and lyophilized.

**Solvent displacement or nanoprecipitation:**

This method is based on the interfacial deposition of polymer followed by displacement of a semi-polar solvent miscible with water from a lipophilic solution. This method involves the use of an organic phase which is completely soluble in the external aqueous phase. The organic solvent diffuses immediately to the external aqueous phase. Therefore, neither extraction nor separation of the solvent is required for the precipitation of the polymer. After the preparation of nanoparticle, the solvent is eliminated and the free flowing nanoparticles are obtained under reduced pressure. This method is used for the drugs that are slightly soluble in water.

**Salting out:**

This method involves incorporation of a saturated aqueous solution of polyvinyl alcohol (PVA) into acetone solution of the polymer under magnetic stirring to form O/W type emulsion. The process differs from nanoprecipitation method in which the polymeric solution is completely miscible with the external aqueous medium. But in this technique, the miscibility of both the phases is prevented by the saturation of external aqueous phase with PVA.

**EVALUATION OF NANOPARTICLES:**

The prepared nanoparticles are evaluated for various parameters like;

1. Yield
2. Drug loading
3. Entrapment efficiency
4. Size and morphology
5. *In vitro* drug release studies
6. Stability testing

**Yield:**

Percentage yield plays a crucial role in determining whether the preparation procedure chosen for incorporating the compound in the polymeric particles is efficient. The percentage yield is expressed as,

$$\text{Yield } (\%) = \frac{\textit{initial amount of raw materials}}{\textit{amount of nanoparticles}} \text{ X } 100$$

The initial amount of raw material represents the amount of active compound and polymer. Certain amount of stabilizing agent or surfactant is being adsorbed during the preparation. Therefore a correction factor should be introduced.

$$\text{Yield } (\%) = \frac{\textit{initial amount of raw materials}}{\textit{amount of nanoparticle } X\left(1-\textit{fraction of residual stabilizing agent}\right)} \text{ X}100$$

**Drug loading:**

The drug loaded or drug content is expressed as;

$$\text{Drug loading} = \frac{amount\ of\ drug \in nanoparticles}{amount\ of\ nanoparticles} \quad X\ 100$$

**Entrapment efficiency:**

Drug entrapment efficiency is the initial amount of drug that is incorporated or adsorbed onto the particles. It is defined as,

$$\text{E.E}\ (\%) = \frac{drug\ loaded}{of\ initial\ content\ X\left(1 - fraction\ of\ residual\ stabilizing\ agents\right)} \quad X\ 100$$

Since the size of the nanoparticles is small, the determination of the drug loading is done after the separation of the free drug from bound drug.

**Size and morphology:**

Scanning electron microscopy (SEM), transmission electron microscopy (TEM) and photon correlation spectroscopy (PCS) are the most commonly used instruments for determining the particle size of nanoparticles.

Scanning electron microscopy is used for the field of nanocarriers. It has high resolution and easy sample preparation. But the sample should withstand high vacuum during analysis. To visualize the particles, they have to be conductive. Therefore, coating of the surface of the sample with gold is required. The thickness of the coating is 20nm. Depending on the amount of the additives, the particles are partially or completely hidden in a matrix of additives. Therefore removal of the stabilizing agents added during the preparation of the particles is essential.

***Invitro* release studies:**

Due to the very small size of the particles, the release rate observed in vivo can differ greatly from the release obtained in a buffer solution. Depending on the type, drug release from nanoparticles takes place through the following processes;

- The solvent may penetrate the nanoparticles and dissolve the drug which then diffuses out into the release medium.
- The drug may release out of the carrier in order to allow complete release from the carriers.

*In- vitro* release kinetics of the drug entrapped in nanoparticles is evaluated by;

I. Dialysis

II. *In situ* method

III. Sample separation techniques

IV. Ultra filtration at low pressure.

**APPLICATIONS OF NANOPARTICLES:**

- Materials: nanoparticles, carbon nanotubes, paints, biopolymer, coating.
- Ceramic based nanoparticles for entrapping therapeutic agent for photodynamic therapy. In this method photosensitive drug/dye is entrapped with ceramic carrier. These ceramic nanoparticles are widely used for skin and therapeutic purpose.
- Nanomedicines: medical devices, nanodrug, tissue engineering etc.
- The thermo sensitive nanoparticles are used for selective release of the content after specific localization.

**INTRODUCTION TO DIABETES MELLITUS:**

**DIABETES MELLITUS: [7]**

Diabetes has emerged as a major health problem in India. The prevalence of diagnosed diabetes has risen dramatically over the past several decades.

Diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar because the pancreas does not produce enough insulin or because cells do not respond to the insulin that is produced.

**CLASSIFICATION OF DIABETES MELLITUS: [8]**

Diabetes mellitus is classified into four categories: type 1, type 2, gestational diabetes and other specific types. The term "diabetes", usually refers to diabetes mellitus. The rare disease diabetes insipidus has similar symptoms to diabetes mellitus, but without disturbances in the sugar metabolism (*insipidus* means "without taste" in Latin) and does not involve the same disease mechanisms.

**Type 1 diabetes mellitus: [9]**

It results from the body's failure to produce insulin and requires the person to inject insulin or wear an insulin pump. This form was referred as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. There is no known preventive measure against type 1 diabetes. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults, but was traditionally termed "juvenile diabetes" because a majority of these diabetes cases were in children.

Type 1 diabetes are accompanied by irregular and unpredictable hyperglycemia, frequently with ketosis, and sometimes serious hypoglycemia including an impaired counter regulatory response to hypoglycemia, occult infection, gastro paresis (which leads to erratic absorption of dietary carbohydrates) and endocrinopathies (e.g., Addison's disease).

**Type 2 diabetes mellitus:**

Type 2 diabetes is the most common type. It results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. This form was referred to as non insulin-dependent diabetes mellitus (NIDDM) or "adult-onset diabetes". In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity. At this stage, hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver.

**Gestational diabetes:**

Gestational diabetes (GDM) occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level. It resembles type 2 diabetes, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2–5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. Untreated gestational diabetes can damage the health of the foetus or mother. Risks to the baby include macrosomia (high birth weight), congenital cardiac and central nervous system anomalies and skeletal muscle malformations. Hyperbilirubinemia may result from red blood cell destruction. In severe cases, perinatal death may occur most commonly as a result of poor placental perfusion due to vascular impairment. A caesarean section may be performed if there is marked fetal distress or an increased risk of injury associated with macrosomia such as shoulder dystocia.

**Other types:**

- Prediabetes is a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 DM.
- Latent autoimmune diabetes of adults (LADA) is a condition in which type 1 DM develop in adults. They are initially misdiagnosed as having type 2 DM, based on age rather than etilogy.
- Some cases of diabetes are caused by the body's tissue receptors not responding to insulin (even when insulin levels are normal, which is what separates it from type 2 diabetes).
- Genetic mutations (autosomal or mitochondrial) can lead to defects in beta cell function. Abnormal insulin action may also have been genetically determined in some cases.
- Any disease that causes extensive damage to the pancreas may lead to diabetes. Diseases associated with excessive secretion of insulin- antagonistic hormones can cause diabetes.

**Table 3: COMPARISON OF TYPE 1 AND 2 DIABETES:**

| Feature | Type 1 diabetes | Type 2 diabetes |
|---|---|---|
| Onset | Sudden | Gradual |
| Age at onset | Mostly in children | Mostly in adults |
| Body habitus | Thin or normal | Often obese |
| ketoacidosis | Common | Rare |
| Auto antibodies | Usually present | Absent |
| Endogenous insulin | Low or absent | Normal, decreased or increased |
| Concordance in identical twins | 50% | 90% |

**Figure 1: SIGNS AND SYMPTOMS:**

The symptoms of untreated diabetes are;

- Loss of weight,
- Polyuria (frequent urination),
- Polydipsia (increased thirst) and
- Polyphagia (increased hunger).

Symptoms may develop rapid in type 1 diabetes, while they usually develop much more slowly or absent in type 2 diabetes.

Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to changes in its shape, resulting in vision changes. Skin rashes occur in diabetes which is known as diabetic dermadromes.

**PATHOPHYSIOLOGY:**

Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells. Therefore, deficiency of insulin or the insensitivity of its receptors plays a major role in all forms of diabetes mellitus.

Insulin is released into the blood by beta cells (β-cells), found in the islets of Langerhans in the pancreas that raises levels of blood glucose, typically after eating. Insulin is used by about two-third of the body's cells to absorb glucose from the blood for conversion to other needed molecules or for storage.

Insulin controls the signal for conversion for internal storage in liver and muscle cells. Lowered glucose levels result both in the reverse conversion of glycogen to glucose when glucose levels fall and in the reduced release of insulin from the β-cells. This is mainly controlled by the hormone glycagon. Glucose produced from internal liver cell stores (as glycogen) re-enters the bloodstream. Muscle cells lack the necessary export mechanism. Normally, liver cells do this when the level of insulin is low.

When the glucose concentration in the blood is raised beyond its renal threshold, reabsorption of glucose in the proximal renal tubuli is incomplete and part of the glucose remains in the urine (glysosuria). This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney which results in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells and other body compartments, causing dehydration and increased thirst.

**TREATMENT:** [10]

All forms of diabetes are treatable since insulin became available in 1921, and type 2 diabetes may be controlled with medications. Insulin and some oral medications can cause hypoglycaemia (low blood sugars), which are dangerous if severe. Both types 1 and 2 are chronic conditions that cannot be cured. Pancreas transplants have been tried with limited success in type 1 diabetes mellitus. Gastric bypass surgery has been successful in many with morbid obesity and type 2 diabetes mellitus. Gestational diabetes usually resolves after delivery.

**Pedro Tartaj**[11] ***et al.,*** (2003) described the synthetic routes for the preparation of magnetic nanoparticles useful for biomedical applications and possible applications of magnetic nanoparticles with special emphasis on showing the benefits of using nanoparticles. The importance of having well-defined synthetic routes to produce materials not only with similar physical features but also with similar crystallochemical.

**Mansoor Amiji**[12] ***et al.,*** (2005) developed thiolated gelatin nanoparticles to enhance the intracellular delivery potential of plasmid DNA using non viral vectors. Thiolated gelatin was synthesized by covalent modification of the primary amino groups of Type B gelatin using 2-iminothiolane (Traut's reagent). Cytotoxicity evaluations carried out by the formazan (MTS) assay showed that the thiolated gelatin prepared with 20 mg and 40 mg of 2-iminothiolane (SHGel-20 and SHGel-40) per gram of gelatin had comparable cell viability profile to that of the unmodified gelatin. The presence of GSH was found to enhance the release by about 40% in case of thiolated gelatin and about 20% in gelatin nanoparticles under similar conditions of temperature and GSH concentrations. Qualitative results showed highly efficient expression of GFP that remained stable for up to 96 h. The results showed that thiolated gelatin nanoparticles would serve as a biocompatible intracellular delivery system that can release the payload in a highly reducing environment.

**Kunihiko Koshiba**[13] ***et al.,*** (2006) demonstrated plasma adiponectin levels increase after the administration of glimepiride. These unique effects would also be expected to improve other adipocytokines and have anti- atherosclerotic action in patients with metabolic syndrome. Thirty-four patients with type 2 diabetes mellitus who were administrated glibenclamide were randomly divided into two groups. The levels of plasma adiponectin, high sensitive-CRP, TNF-, interleukin-6, homeostasis model assessment-insulin resistance (HOMA-IR), brachial-ankle pulse wave velocity (baPWV) and augmentation index (AI) were measured. HOMA-IR in the GP group was significantly decreased compared to the GB group. Plasma adiponectin levels were significantly increased in the GP group but not in the other groups. Glimepiride appears to improve insulin resistance andatherosclerotic disorders.

**Catarina Pinto Reis**[14] ***et al.,*** (2006) evaluated polymeric nanoparticles have been extensively studied as particulate carriers in the pharmaceutical and medical fields, because they show promise as a drug delivery system as a result of their controlled sustain release properties, sub-cellular size and biocompatibility with tissue and cells. Several methods to prepare nanoparticles have been developed during the last two decades, classified according to whether the particle formulation involves a polymerisation reaction or arises from a

macromolecule or performed polymer so as to facilitate selection of an appropriate nanoencapsulation method according to a particular application.

**H. O. Ammar**[15] ***et al.,*** (2007) improved the solubility and dissolution of glimepiride through complexation with dimethyl-β-cyclodextrin (DM-β-CD). An inclusion complex of glimepiride in DM- β-CD was prepared in a molar ratio of 1: 2 by the kneading method. The drug-CD complex was characterized by scanning electron microscopy, thermogravimetric analysis and X-ray diffractometry. Ternary systems of the drug, cyclodextrin and a water soluble polymer (HPMC, PVP, PEG4000 or PEG6000) were also prepared. The solubility of the drug increased with the addition of cyclodextrin. Dissolution of the drug from the prepared ternary systems was highly dependent on the polymer type and concentration. Dissolution of the drug from ternary systems containing PEG4000 or PEG6000 seemed to be generally higher than from systems containing HPMC or PVP. This should help improve the biological performance of the drug.

**Manivannan Rangasamy**[16] ***et al.,*** (2008) developed β-Cyclodextrin complexation of meloxicam to enhance the solubility features of the drug. The possibility of developing meloxicam tablets are allowing to get fast reproducible and complete drug disintegration by using β-Cyclodextrin complexation. The tablets were prepared by direct compression technique and evaluated for thickness, uniformity of weight, content uniformity, hardness, friability, disintegration and in-vitro dissolution time. Stability study for the selected formulations was conducted for a period of one month at 25˚C, 40˚C and 60˚C respectively and the formulations exhibited no alteration in the physical appearance or content of the tablet. The complex prepared by direct compression method was found to be yield very reliable and best results over that of the wet granulation method.

**V. G. Kuchake**[17] et al., (2009) studied the effect of metformin when it is given in combination with either glimepiride or glibenclamide on glycaemic control in patient with type 2 Diabetes Mellitus. Patients were randomly assigned for treatment based on metformin-glibenclamide 1000/10 mg tablets or metformin-glimepiride1000/2mg for 12 weeks. The comparisons were conducted between these two groups for HbA 1C, FPG, PPG and lipid profile. Significant reductions in HbA1c were found in both groups but the patients treated with metformin-glimepiride resulted in significantly greater reductions in HbA 1C (-1.4%) than metformin-glibenclamide (-1.2%). Metformin-glimepiride tablets resulted in significantly greater reductions in HbA 1C and fasting plasma glucose compared with metformin plus glibenclamide in patients with type 2 diabetes mellitus.

**S. Tamizhrasi**[18] ***et al.,*** (2009) prepared and evaluated polymethacrylic acid nanoparticles containing lamivudine in different drug to polymer ratio by nanoprecipitation method. SEM indicated that nanoparticles have a discrete spherical structure without aggregation. The particle size of the nanoparticles was gradually increased with increase in the proportion of polymethacrylic acid polymer. The drug content of the nanoparticles was increasing on increasing polymer concentration up to a particular concentration. The in-vitro release behaviour from all the drug loaded batches was found to be zero order and provided sustained release over a period of 24 h. The developed formulation overcome and alleviates the drawbacks and limitations of lamivudine sustained release formulations and could possibility be advantageous in terms of increased bioavailability of lamivudine.

**Vipul P. Patel**[19] ***et al***., (2009) prepared the solid inclusion complexes of glipizide and β-CD at a molar ratio of 1:1 and 1:2 by mixing, kneading, and co-precipitation methods both on small and large scales. The effect of parameters such as kneading time and temperature on complexation was also studied. In vitro release studies were carried out in phosphate buffer (pH 7.4). All the methods of preparation of complexes were found to be useful in increasing the solubility of glipizide except mixing method where the rise in solubility was not significant. Both kneading and co-precipitation methods in 1:2 molar ratios were found to be equally effective in improving the solubility of glipizide. The formation of inclusion complexes was evident in these formulations as shown by IR and XRD studies. But when carried out on a large scale, co-precipitation method was found to be more tedious and time-consuming than kneading method. Moreover percent recovery of complexes in the kneading method was found to be 98.76% as compared to 92.05% in case of co- precipitation method. Inclusion complexes prepared by kneading method in 1:2 molar ratios were suitable for improving the solubility of glipizide. The same formulation was prepared at large scale and optimum formulation conditions were established.

**N.Arunkumar**[20] ***et al.,*** (2009) improved the solubility and dissolution characteristics of a poorly soluble drug (atorvastatin calcium) using nanosuspension technology. Nanoparticles were characterized in terms of size and morphological characteristics. Saturation solubility and dissolution characteristics were investigated and compared to the commercial drug. Crystallinity of the drug was also evaluated by performing thermal gravimetric analysis (TGA), differential scanning Calorimetry (DSC) and powder X-ray diffraction (PXRD). It has been shown that the crystalline state of the drug is reduced following particle size

reduction and the dissolution rates of amorphous atorvastatin calcium nanoparticles were highly increased in comparison with commercial drug by the enhancement of intrinsic dissolution rate and the reduction of particle size, resulting in an increased specific surface area.

**L. Zhang**[21] ***et al.,*** (2010) developed nanoparticle systems for antimicrobial drug delivery. Even though the therapeutic efficacy of the drugs has been well established, inefficient delivery could result in inadequate therapeutic index and local and systemic side effects including cutaneous irritation, peeling, scaling and gut flora reduction. Nanostructured biomaterials, nanoparticles in particular, have unique physicochemical properties such as ultra small and controllable size, large surface area to mass ratio, high reactivity, and functionalizable structure. These properties can be applied to facilitate the administration of antimicrobial drugs, thereby overcoming some of the limitations in traditional antimicrobial therapeutics. In recent years, encapsulation of antimicrobial drugs in nanoparticle systems has emerged as an innovative and promising alternative that enhances therapeutic effectiveness and minimizes undesirable side effects of the drugs.

**Camelia Nicolescu**[22] ***et al***., (2010) evaluated the possibilities to improve Repaglinide - an oral antidiabetic drug -solubility in water, based on inclusion complexes formation with β-cyclodextrin (β-CD), hydroxypropyl β-cyclodextrin (HP-β-CD) and randomly methylated β-cyclodextrin (RAMEB), respectively, and also to estimate their composition and apparent stability constants. We have noticed that the phase solubility diagram for the repaglinide – HP-β-CD inclusion complex is a type, while those of repaglinide - β-CD and RAMEB are B type. The phase-solubility diagrams indicate an enhancement of the repaglinide solubility in the presence of β-CD, as well as its derivatives, HP-β-CD and RAMEB in different extents, related to the type of cyclodextrin.

**Rezaei Mokarram**[23] ***et al.,*** (2010) prepared indomethacin nano-solid suspension in a polymeric matrix by controlled precipitation method to increase the solubility and rate of the dissolution of poorly soluble model drug. It is characterized by differential scanning calorimetry (DSC), X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and evaluated for in vitro solubility and dissolution rate. Absence of thermal and diffractional peaks in DSC and XRD studies indicated that indomethacin interacts with PVP in solid phase. It was found that particle size distribution depend to the polymer MW and drug: polymer ratios. Spectroscopy methods and Transmission Electron Microscopy (TEM)

images showed that indomethacin dispersed as amorphous nanosize particles in freeze dried powder.

**Mitra Jelvehgari**[24] ***et al.,*** (2010) formulated and evaluated effect of the microencapsulation of theophylline loaded nanoparticles on the reduction of burst release. Nanoparticles were prepared by using water-in-oil-in-water (W1/O/W2 double-emulsion solvent diffusion/evaporation method), taking different ratios of drug/polymer. Solvent systems consist of ethyl acetate and dichloromethane for microspheres and nanospheres, respectively. In the current study formulations were characterized by loading efficiency, yield, particle size, zeta potential, X-ray diffraction (XRD) and differential scanning calorimetry (DSC). The burst was significantly lower with composite microparticles and may be explained by lower diffusion of the drug from double polymeric wall formed by the nanoparticles matrix followed by another diffusion step through the microparticle polymeric wall.

**Partha Saha**[25] ***et al.,*** (2010) performed ampicillin trihydrate-loaded chitosan nanoparticles by modified ionic gelation method and evaluated their antimicrobial activity. Parameters such as the zeta potential, polydispersity, particle size, entrapment efficiency and in vitro drug release of the nanoparticles were assessed for optimization. Scanning electron microscopy revealed that the nanoparticles were in the nano size range but irregular in shape. Concentrations of 0.35 %w/v of chitosan and 0.40 %w/v sodium tripolyphosphate (TPP) and a sonication time of 20 min constituted the optimum conditions for the preparation of the nanoparticles. The nanoparticles demonstrated superior antimicrobial activity to plain nanoparticles and the reference, due probably to the synergistic effect of chitosan and ampicillin trihydrate.

**S. Debnath**[26] ***et al.,*** (2010) prepared cytarabine loaded nanoparticles by ionotropic gelation. This was characterized by SEM and was found to be in the range of 200nm. The mechanism by which the drug is being released is non- Fickian (anomalous) solute diffusion mechanism. It is evident from the result that initial burst release was retarded or delayed due to absorption of coating material. The in vivo bio distribution study results showed that the nanoparticles were having better distribution of drug compared to free drug in different organs like spleen, lungs, kidney etc.

**C. Rubina Reichal**[27] ***et al.,*** (2011) developed floating matrix tablets of glimepiride to prolong the gastric residence time and thereby increase drug bioavailability. Diabetes condition influences the gastric emptying time which affect the absorption of the drug. The

tablets were prepared by direct compression technique, using various grades of rate controlling polymers, Carbopol 934P either alone or in combination and other standard excipients. Tablets were evaluated for physical characteristics viz. hardness, % friability, floating capacity and content of dosage form. Floating matrix tablets based on the combination of polymers exhibited desired floating and prolonged drug release for 8h.

**Narasimha Reddy**[28] ***et al.,*** (2011) investigated the release of Glimepiride and Parecoxib from the dosage form at a particular site and controlling the release of drug from the dosage form and achieving controlled plasma level of the drug as well as improving bioavailability. The tablets were prepared to achieve controlled plasma level of the drug which is especially in diabetes mellitus patients with pain therapy. The tablets were prepared by direct compression technique. Both the drugs were found compatible with the excipient used. All the formulations were found to have good pre compression and post compression parameters. The optimized formulation was subjected to accelerated stability studies.

**Neha Chowdhary**[29] ***et al.,*** (2011) developed to improve the solubility and dissolution rate of poorly water soluble glimepiride by complexation with HP-β-cyclodextrin and impart a fast release in a single formulation. The complexes of glimepiride with HP-β-cyclodextrin were physical mixing, co-grinding and kneading methods and were characterized and evaluated to study the effect on complexation in dissolution and solubility profiles. Remarkable improvement was observed in the invitro drug release profile in 0.1N HCl and phosphate buffer pH 6.8 with all complexes. The characterization studies confirmed that the inclusion of glimepiride with the non-polar cavity of HP-β-CD. The glimepiride: HP-β-CD (1:3 M) complex prepared by kneading method exhibited higher dissolution rate and dissolution efficiency value in pH 6.8 phosphate buffer than pure drug and physical mixture. The orodispersible tablets were formulated using the kneaded complex with suitable excipients showed 100% release within 5 mins. Stability studies were carried out as per ICH guidelines indicated that there was no significant change found in physical appearance, degradation time and wetting time of the tablets.

**Veerendra S. Rajpurohit**[30] ***et al.,*** (2011) prepared solid dispersions of glimepiride by modified solvent fusion method using PEG 6000 and PVP K25 (as carrier). Invitro release was carried out using USP II dissolution apparatus. Multilinear regression analysis was applied to develop mathematical model to estimate cumulative drug release. Improvement in dissolution behaviour of solid dispersion batches was observed due to conversion of crystalline form of drug to amorphous form as confirmed by DSC, FTIR studies and X-RD

studies. SEM photographs showed porous nature of particle surface. Uniformity of content of different batches was found to be in range as specified by IP. Solid dispersion prepared via modified fusion solvent method was proved to be beneficial in enhancement of dissolution rate of poorly-water soluble drug using hydrophilic carriers. Retrospectively, this model can further be utilized to design solid dispersions for desired release characteristics.

**Shahrooz Saremi**[31] ***et al.,*** (2011) prepared and evaluated mucoadhesive core-shell nanoparticles based on copolymerization of thiolated chitosan coated on poly methyl methacrylate cores as a carrier for oral delivery of docetaxel. It is prepared by a radical emulsion polymerization method using cerium ammonium nitrate as an initiator. Nanoparticles were spherical with mean diameter below 200 nm, polydispersity of below 0.15, and positive zeta potential values. In vitro release studies showed a sustained release characteristic for 10 days after a burst release at the beginning. Ex vivo studies showed a significant increase in the transportation of docetaxel from intestinal membrane of rat when formulated as nanoparticles. It can be concluded that by combining the advantages of both thiolated polymers and colloidal particles, these nanoparticles can be proposed as a drug carrier system for mucosal delivery of hydrophobic drugs.

**Durga Prasad Pattanayak**[32] ***et al.,*** (2011) designed bilayer tablet formulation consisting of two drug containing layers which comprises Metformin sustained release layer and an immediate release layer of Glimepiride was optimised separately and constituted in bilayer tablet, a common analytical method for quantitative combined drug estimation was employed and evaluated. Two different matrix formulations were developed, one matrix layer with hydrophilic swellable polymer and another with hydrophobic polymer as carriers for sustained drug delivery from matrices and were evaluated. Hydroxypropyl methyl cellulose and Polyethylene oxide was used as polymers in order to get the sustained release profile over a period of 24 h. Stability of the drug release profiles at 6 months in $4^{00}$ C and 75%RH suggesting that HPMC based sustained release formulation was stable than the Polyethylene oxide sustained release formulation due to its stable and better targeting profile in terms of drug release. This formulation also exhibited the best fitted formulation into zero order kinetics and non-Fickian transport of the drug from the tablets was confirmed. Bilayer tablet prepared from optimised formula was found to be best suited method for fixed dose combination of sustained release Metformin HCl and immediate release Glimepiride.

**Adel M. Aly**[33] ***et al.,*** (2011) prepared Glimepiride rapidly disintegrating tablets by direct compression and evaluated Pharmaburst as a newly introduced diluent for this type of tablets,

either alone or in combination with other well known tablet excipients. Pharmaburst alone is sufficient to produce rapidly (orally) disintegrating tablets of Glimepiride with good physical characteristics, better compatibility and shorter in-vivo and in-vitro disintegration time. The prepared Glimepiride RDT was found to have faster onset of action than the conventional Glimepiride tablets. Glimepiride RDT containing Pharmaburst alone were found to be stable when subjected to accelerated stability conditions (40 °C / 75 % relative humidity) for at least 3 months.

**Anilkumar J. Shinde**[34] ***et al.,*** (2011) formulated nanoparticles for simvastatin drug. Since simvastatin undergoes extensive first pass extraction in the liver, the availability of the drug to the general circulation is low (< 5%). Nanoparticles were prepared by precipitation-solvent deposition method using $3^2$ full factorial designs. The prepared formulations were further evaluated for drug content, in vitro drug release pattern, and short term stability and drug excipient interactions. Drug: polymer ratio and concentration of stabilizer were found to influence the particle size and entrapment efficiency of simvastatin loaded PLGA nanoparticles. The release was found to follow first order release kinetics with fickian diffusion mechanism for all batches. These results indicate that simvastatin loaded PLGA nanoparticles could be effective in sustaining drug release for a prolonged period.

**Subhranshu Panda**[35] ***et al.,*** (2011) prepared solid dispersions by solvent evaporation technique to enhance solubility. Different ratios of PEG 6000 to Glimepiride were taken for solid dispersion. The Films of Glimepiride solid dispersion equivalent to 2mg Glimepiride , were developed by solvent casting method using different Polymers, HPMC K4M, Sodium CMC, carbopol 971P and polyox. The prepared mucoadhesive buccal patches were evaluated for Swelling index, Residence time, Folding endurance, Tensile strength and Mucoadhesive strength. In vitro release was carried out in simulated saliva solution using modified USP type II apparatus at 50 rpm. Ex vivo release studies were performed with few selected batches and its results along with evaluation parameter were taken in to account to select optimized batch. The release of Glimepiride from developed formulations was found to be fickian diffusion controlled. A Short- term accelerated stability study was carried out for one month and the formulation found stable for that period of time.

**Chandra Sekhar Y**[36] ***at el.,*** (2011) prepared glimepiride sustained release matrix tablet system by using natural polymers carrgeenan and xanthan gum. The sustained release matrix tablets of glimepiride were prepared by wet granulation method varying the concentrations of polymers. The formulated granules were evaluated for angle of repose, bulk density, tapped

density, Carr's index and hausner's ratio. The formulated tablets were evaluated for uniformity weight, hardness, friability, and drug content, invitro swelling studies, invitro dissolution study and kinetic data analysis. The obtained results were clearly indicating that the formulated tablets results are within the range. The release of drug was anomalous non-Fickian transport of diffusion from zero order release from the formulation was observed.

**Khushbu Shenghani**[37] ***et al.,*** (2012) developed Bi-layer tablets containing two layers, one the immediate release and one containing the extended release layer. Extended layer were prepared by wet granulation method using different viscosity grade of Hydroxypropyl methylcellulose (HPMC K100M and HPMC K200M) as polymers and immediate release layer were prepared by wet granulation method using superdisintegrant such as sodium starch glycolate and crosscarmellose sodium. Binder used was polyvinyl pyrollidone (PVP K-30). The tablets were then evaluated for the various physicochemical parameters and for the dissolution profile and then the prepared batches were compared with the Reference product. The present study concluded that Bilayer tablets of Metformin Hydrochloride as an extended release layer and Glimepiride as an alternative to the immediate release conventional dosage form.

**Mohammed S. Khan**[38] ***et al.,*** (2012) developed and evaluated mucoadhesive nanoparticles of chitosan using Tramadol HCl. Spray drying method was employed for producing nanoparticles using different drug to polymer ratio. Nanoparticles were evaluated for variables like yield, drug loading, entrapment efficiency, swelling, in-vitro mucoadhesion, particle size, polydispersity index & zeta potential, scanning electron microscopy, transmission electron microscopy, X-ray diffraction study and drug polymer compatibility by Differential scanning calorimetry & Fourier Transform Infrared Radiation studies. From the above studies it is considered that Tramadol HCl loaded chitosan nanoparticles is a promising delivery through nasal route for relief of pain.

**Lakshmi Sirisha Kotikalapudi**[39] ***et al***., (2012) prepared solid lipid nanoparticles of Domperidone. DOM loaded SLN were prepared by hot homogenization followed by ultrasonication technique. DOM- SLN were characterized for particle size, polydispersity index (PDI), zeta potential and entrapment efficiency and invitro drug release behaviour were investigated. P-XRD and DSC analysis were performed to characterize the state of drug and lipid modification. Shape and surface morphology were determined by transmission electron microscopy (TEM). SLN formulations were subjected to stability study over a period of 30 days. Invitro release studies demonstrated that the SLN formulation possessed a controlled

release over a period of 48 hrs. Fairly spherical shaped, stable and controlled release DOM-SLN could be prepared by hot homogenization followed by ultrasonication technique.

**Ghanshyam Patel**[40] ***et al***., (2012) formulated Pharmaceutical equivalent bilayer tablet of Anti-diabetic by using the wet granulation method. In this formula one layer provide the loading dose by immediate release and another layer provide maintenance dose up to 10 hrs by extended release. The drug excipients compatibility study was carried out with FTIR study. Immediate release fraction was formulated by using cross carmellose sodium (CCS) as a disintegrating agent and sustained release fraction was formulated by using hydroxy propyl methyl cellulose (HPMC) as a rate controlling polymer. The granules were evaluated for angle of repose, bulk density, tapped density and compressibility index showed satisfactory results. The prepared bilayer tablets were evaluated as thickness, hardness, friability and in-vitro release studies. In-vitro dissolution study was carried out for 10 hrs using USP dissolution apparatus type II with pH phosphate buffer as dissolution medium. Stability study was carried out for the optimized formulation at 40°C/75% RH shows that there was no significant change in physical and chemical parameter of the tablet.

**Naik J B**[41] ***et al***., (2012) formulated and optimized Repaglinide (Rg) loaded Eudragit RL-100 nanoparticles as a sustained release carrier. It is developed by High Pressure Homogenizer Emulsification (HPHE) -Solvent Evaporation method in different ratios. The method was optimized using design of experiments by employing a 3-factor, 3-level Design Expert (version 8.0.7.1) Statistical Design Software and was subjected to various studies for characterization including Transmission electron microscopy, X-ray diffraction, Encapsulation efficiency, Particle Size Analysis. These studies are favourably revealed that the mean particle diameter of optimized formulation was 50 nm with crystalline nature. Moreover, formulated nanoparticles were also subjected to Fourier Transform Infrared Spectroscopy, Differential Scanning Calorimetry for interaction between drug and polymer. The results were positive. Hence, the designed system could possibly be advantageous in terms of sustained release drug delivery system, to achieve reduced dose frequency, side effects as well improvement in patient compliance.

**Madhusudhanareddy Induri**[42] ***et al.,*** (2012) developed and validated an analytical method for quantitative determination and dissolution studies of Glimepiride in tablets. Glimepiride shows absorption maxima at 225 nm and obeyed Beer's law in the range of 6.0 – 14.0 µg/mL. Percentage recovery of glimepiride for the proposed method ranged from 99.32 to 100.98% indicating no interference of the tablet excipients. It was concluded that the

proposed method is simple, easy to apply, economical and used as an alternative to the existing spectrophotometric and non-spectrophotometric methods for the routine analysis of glimepiride in pharmaceutical formulations and in vitro dissolution studies.

**Mohd Abdul Hadi**[43] ***et al.,*** (2012) developed sustained release tablets of glimepiride by wet granulation method based on combination of hydrophilic (HPMC15cps, HPC) and hydrophobic (Ethyl cellulose) polymers. The drug excipients mixtures were subjected to pre-formulation studies, physicochemical studies, in- vitro drug release, kinetic studies and stability studies. FTIR and DSC studies shown there was no interaction between drug and polymers. The physicochemical properties of tablets were found within the limits. The drug release from the optimized formulation was extended for a period of 12 hrs. The kinetic treatment showed that the release of drug follows first order models. The optimized formulations were subjected to stability studies and shown there were no significant changes in drug content, physicochemical parameters and release pattern. Results of the present study indicated the suitability of the above mentioned polymers in the preparation of sustained release formulation of glimepiride.

**Wagh V. T.**[44] ***et al.,*** (2012) enhanced the solubility of Glimepiride by using solid dispersion technique. The polymers used were Poloxamer 188 and Poloxamer 407 and solid dispersions were prepared by kneading method. The solubility study was carried out to study the effect of polymers on solubility of Glimepiride. The prepared solid dispersions were characterized by In-vitro solubility Study, %Drug content; Fourier transforms spectroscopy (FTIR), In vitro drug dissolution to identify the physicochemical interaction between drug and excipients. The dissolution studies of solid dispersion were performed by using USP II apparatus. The solid dispersion prepared with Poloxamer 188 showed better drug release as compared to solid dispersion prepared with Poloxamer 407. Tablet formulations were prepared by direct compression technique and developed tablet formulations were evaluated for various pharmaceutical characteristics viz. hardness, % friability, weight variation, drug content, in-vitro dissolution profiles. Thus Results showed that the solid dispersion technique by using poloxamer successfully used for enhancing the solubility of Glimepiride.

**Neha Pachisia**[45] ***et al.,*** (2012) prepared controlled transdermal systems of glimepiride were prepared using natural polymer chitosan for the extended and controlled delivery of the drug. Optimization of the system was done using in vitro drug permeation studies through rat skin. Blood glucose reducing hypoglycemic activity of the systems was studied. The in vitro permeation rate across the rat skin varied with the varying drug: polymer ratio in the patch.

The present study demonstrates that this novel matrix controlled transdermal delivery system exhibited better control of diabetes than conventional oral route.

**Hari Har Prasad.M**[46] ***et al.,*** (2012) aimed at developing a Glimepiride immediate release tablet formulation for the effective treatment of Type-2 Diabetes mellitus (or) Non-Insulin-Dependent Diabetes Mellitus (or) adult-onset diabetes. To provide the patients with the most convenient mode of administration, there was a need to develop immediate release dosage form, particularly one that disintegrates rapidly and disperses and helps in enhancing the Bioavailability of the drug. Glimepiride immediate release tablets were formulated by using wet granulation method and Povidone k 30, starch as binders, croscarmellose sodium, Sodium Starch Glycolate, Crosspovidone as disintegrants, Lactose monohydrate as Diluent and Magnesium stearate as Lubricant. The tablets were evaluated for Pre compression and Post compression Parameters after conducting preformulation Studies. All the parameters were within the pharmacopoeial limits and the drug disintegration time was less and the Invitro dissolution studies showed that the drug release was fast in Formulation containing Sodium Starch Glycolate as Super disintegrant and Povidone k 30 as binder.

**Patel Naveen**[47] ***et al.,*** (2012) designed sustained release mucoadhesive buccal patch formulation of Glimepiride in order to by-pass GIT and release the drug for extended periods of time. Glimepiride is a potent drug against Diabetes mellitus-II with a half life of 3-5 hours. Though Glimepiride has 100% oral absorption, due to high first pass metabolism its bioavailability is less. The recommended dosage is 4 mg b.i.d leading to administration of the drug twice a day. Buccal patches were formulated using polymers Carbopol 934 P (CP 934 P), Ethyl Cellulose (EC) and Hydroxy Propyl Methyl Cellulose (HPMC) in various proportions and combinations. Tween 80 was used as permeation enhancer and glycerine as plasticizer. The patches were prepared by solvent casting method. The designed patches were evaluated for thickness uniformity, folding endurance, weight uniformity and content uniformity and swelling behaviour. In vitro diffusion studies were conducted for 24 hours in phosphate buffer (pH 6.6) solution using dialysis membrane. Formulation containing maximum amount of swellable and hydrophilic polymer HPMC K100M and CP 934 P, showed higher swelling index and could sustain for 24 hours. This occurred due to more hydrophilic polymeric matrix composition which retarded the release of the drug.

**Sreenivasa Rao K**[48] ***et al.,*** (2012) prepared and characterized inclusion complexes of Glimepiride with β-CD and HP-β-CD. The phase solubility analysis indicated the formation of 1:1 molar inclusion complex of Glimepiride with β-CD and HP-β-CD. Apparent stability

constant (KC) was 32.95 M-1 and 42.57 M-1 for β-CD and HP-β-CD complexes respectively. The inclusion complexes were prepared by three different methods viz. Physical, Kneading and Co-precipitation method. The prepared complexes were characterized using FT-IR, and Differential Scanning Colorimetry (DSC). The inclusion complex prepared with HP-β-CD by Kneading method exhibited greatest enhancement in solubility and fastest dissolution (97.41% GMP release in 60 min) of GMP.

**P. Srinivas**[49] ***et al.,*** (2012) prepared controlled release formulation of Moxifloxacin hydrochloride ocular nanoparticles by solvent displacement method using Eudragit RL 100 as a polymer. Different formulations were prepared by varying the ratios of drug and polymer and varying the ratios of organic and aqueous phase. The formulations were evaluated in terms of particle size, FTIR, drug entrapment efficiency and in vitro drug release profile. The anti bacterial activity against gram positive and gram negative bacteria were determined. In vivo studies were carried out by Draize test. The mean particle size for drug loaded formulations was found to be below 200 nm. The zeta potential remained in the range of positive values for all batches +10 mV to +40mV. The formulation possesses good antibiotic activity against Escherichia coli, Bacillus subtilus and Staphylococcus aureus microorganism and no eye irritation on in-vivo testing.

**Jaimin D patel**[50] ***et al.,*** (2012) prepared methotrexate loaded ethyl cellulose nanoparticles using solvent evaporation method with PVA as dispersion medium. Nanoparticles with varying drug: polymer ratio and dispersion medium were prepared by using 32 factorial designs. Prepared nanoparticles were evaluated with respect to particle size, drug entrapment efficiency and in vitro release study. In-vitro release pattern of Methotrexate from Nanoparticles in the phosphate buffer of pH 7.4, showed a biphasic pattern with an initial burst effect and prolonged release over 24hrs. Stability studies showed that similar drug content and closest in vitro release profile to initial data when the sample stored at 250C.

**Shantanu Tamuly**[51] ***et al.,*** (2012) prepared calcium phosphate nanoparticles with bovine serum albumin using different stirring times and each analysed for entrapment efficiency. The void calcium phosphate nanoparticles were injected intramuscularly into rats for testing the site-specific inflammation. The muscle samples were collected on the 14th day of injection. The smallest particle size of about 40 μm of calcium phosphate nanoparticle–BSA complex was obtained by stirring for 1 h. The void calcium phosphate nanoparticles did not elicit any site-specific reaction in rats, as revealed by in histopathological examination.

Hence calcium phosphate nanoparticles can be efficiently used as an adjuvant for non-live vaccines.

**Swati Srivastava**[52] ***et al.,*** (2012) compared the efficacy and safety of sitagliptin and glimepiride in treatment of patients with type 2 diabetes mellitus inadequately controlled with metformin alone. In an 18 week, randomized parallel group interventional trial, 50 subjects who were only on metformin as antidiabetic agent, with inadequate glycaemic control, were randomized to either sitagliptin 50/100mg or glimepiride 1/2 mg per day. Dose of drugs was adjusted after 4 weeks if glycaemic control was not reached. At 18 weeks both groups (sitagliptin and glimepiride) produced significant ($P<0.001$) reduction in hbA1c (-0.636% and -1.172% respectively), with 12% patients in sitagliptin group and 36% patients in glimepiride group achieving target hbA1c. Reduction was also significant ($P<0.001$) in both groups in FPG (-15.49mg and -29.84mg respectively) and 2hPPG (-34.28mg and -44.83mg respectively). Sitagliptin group showed net decrease in bodyweight by 0.102kg whereas glimepiride group showed net increase in body weight by 0.493 kg. Incidence of hypoglycemia was 4% in sitagliptin group and 8% in glimepiride group. In this study addition of sitagliptin and glimepiride to metformin monotherapy, produced significant improvement in glycaemic control. Benefits were more with glimepiride in comparison to sitagliptin. Sitagliptin was well tolerated, with lower risk of hypoglycemia than glimepiride, and produced weight loss as compared to weight gain with glimepiride.

**Hindustan Abdul Ahad**[53] ***et al.,*** (2012) developed matrix tablets of glimepiride with Hibiscus rosa-sinensis leaves mucilage and Povidone and to study its functionality as a matrix forming agent for sustained release tablet formulations. Mucilage from Hibiscus rosa-sinensis was extracted, isolated, purified and characterized. Physicochemical properties of the dried powdered mucilage of Hibiscus rosa-sinensis leaves were studied. Various formulations of glimepiride Hibiscus rosa-sinensis leaves mucilage and Povidone were prepared. The formulated tablets were tested for mechanical properties, friability, swelling behaviour, invitro drug release pattern and the dissolution data were subjected to mathematical modelling and the optimized formulation were tested for accelerated stability studies. The formulated tablets were found to have good mechanical properties, good swelling properties. The invitro dissolution data was fitted to zero order and the release of the drug from the formulation followed Higuchi's release. The accelerated stability studies revealed that the tablets retain their characters even after stressed storage conditions. From this study it was concluded that the dried Hibiscus rosa-sinensis leaves mucilage and

Povidone combination can be used as effective matrix forming material for making sustained release matrix tablets of glimepiride.

**Shirse Prabhakar**[54] ***et al.,*** (2012) enhanced the solubility and dissolution of the drug by preparing its complex with β-cyclodextrin and Hydroxypropyl beta cyclodextrin-β-cyclodextrin. In this present study attempts has been made to prepare, formulate and characterize inclusion complexes of glimepiride with β-cyclodextrin and Hydroxypropyl beta cyclodextrin-β-CD. The inclusion complexes were prepared by three different methods viz. Physical, kneading and co-precipitation methods. The inclusion complex containing glimepiride: β-CD and Hydroxypropyl beta cyclodextrin-β-CD were further formulated into fast dissolving tablets(FDT) by direct compression technique using super-disintegrants like crosscaramelose sodium, crosspovidone and sodium starch Glycolate were used. The prepared complexes were characterized using DF-IR and differential scanning calorimetry and finally the prepared fast dissolving tablets were evaluated for various pharmaceutical characteristics viz. Hardness, %friability, weight variation, wetting time, drug content and invitro dissolution profiles. The results of stability studies revealed to change in physical appearance, hardness, drug content and invitro dissolution profile, thus indicating that formulation was stable.

**Prabhakar Shirse**[55] ***et al.,*** (2012) formulated and evaluated the bilayered tablets containing immediate release layer of HP-β-Cyclodextrin inclusion complexed Glimepiride to produce immediate therapeutic effect and sustained release layer containing Metformin Hcl by using HPMC as release retardant. The reason for Bi- layer tablet formulation is to separate physically or chemically incompatible ingredients and to produce repeat action or prolonged action tablet. To optimize and develop a robust and stable formulation, both wet & dry granulation processes were used for formulation. The compressed tablets were evaluated for physico-chemical properties. The stability studies of the products also comply with ICH guidelines. FTIR studies clearly indicate that there is no drug polymer interaction. This formulation also exhibited the best fitted formulation into zero order kinetics and non-Fickian transport of the drug from the tablets was confirmed. The present study concluded that bilayer tablets of Glimepiride & Metformin Hcl shall be a good method to improve bioavailability of drugs.

**Kishore K**[56] ***et al.,*** (2013) prepared solid dispersion of Glimepiride to enhance the solubility. It is prepared by dissolving drug and polyvinyl pyrrolidine K30 in dichloromethane and the solvent is removed by rotary evaporator under reduced pressure. The solubility increased

around twenty times greater when drug and carrier is used in1:10 ratios. The Oro dispersible tablets were prepared by using Sodium starch glycollate, cross caramellose sodium, pregelatinized starch and polacrilin potassium as super disintegrants. The rapid disintegration is obtained to polacrilin potassium (10%) and maximum drug release obtained in 10 min. From these results it is concluded that solubility of glimepiride is increased by preparing solid dispersion and rapid bioavailability is observed by preparing orodispersible tablets.

**M. Tejakrishna**[57] ***et al.,*** (2013) developed sustained release of Glimepiride to enhance the gastrointestinal residence time, for this purpose mucoadhesive micro beads were formulated by employing Ionic gelation method with HPMC and Na CMC as coating polymers. Formulated mucoadhesive micro beads were properly evaluated for size distribution, tapped density entrapment efficiency, wall thickness, drug release studies, SEM and GI residence time. The rate of drug release was found to be decreased by increasing the concentration of the coat polymer. The rate of drug release was found to be less for mucoadhesive micro beads formulated by Na CMC than compared to mucoadhesive micro beads formulated by HPMC. The mucoadhesive micro beads prepared with HPMC and Glimepiride in 1:9 ratio shown prolonged drug release up to 12 hours. The release follows first order kinetics and mechanism of drug release was found to be governed by diffusion mechanism.

**Ram Alpana**[58] ***et al.,*** (2013) prepared Solid dispersions of tolbutamide with urea, sorbitol and mannitol by fusion method with a view to increase its water solubility. The major problem with this drug is its very low solubility in biological fluids which results in poor bioavailability after oral administration. Therefore Solid dispersions of tolbutamide with urea, sorbitol and mannitol were prepared by fusion method with a view to increase its water solubility. The equilibrium solubility study showed that the solubility of tolbutamide was enhanced 3.90 times in the prepared urea solid dispersion.

**Yashavantsinh Chavd**[59] ***et al.,*** (2013) formulated and evaluated lamivudine microspheres using a combination of eudragit RS 100 and eudragit RL 100 polymers. The lamivudine microspheres were prepared by the solvent evaporation method using different concentration of the Eudragit polymers. The effect of polymer ratio on % drug encapsulation efficiency was investigated using 3 full factorial designs. The parameters determined were bulk density, tape density, angle of repose, particle size, drug content, % drug encapsulation efficiency & in vitro dissolution. As the stirring speed increased, the particle size decreased and as the concentration of eudragit increases, the particle size also increased. Larger microspheres showed greater drug loading and smaller microspheres showed a faster drug release.

**Vyjayanthimala**[60] ***et al.,*** (2014) prepared chitosan Nanoparticles of Zidovudine for antiviral therapy by emulsion droplet coalescence method. The concentration of the polymer Chitosan was selected based on the results on preliminary screening. The nanoparticles prepared were evaluated for morphology, loading efficiency and in vitro release. The particle shape and morphology of the prepared Zidovudine nanoparticles were determined by SEM analysis. The amount of Zidovudine entrapment in the nanoparticles was calculated by the difference between the total amount of drug added to the nanoparticle and the amount of non-entrapped drug remaining in the aqueous supernatant. A Franz diffusion cell was used to monitor Zidovudine release from the nanoparticles. The percentage cumulative drug release after 12 hours was 75.54% and 75.89% respectively. However about 15% initial burst release was found at 1 hour in all formulations. In-vitro antiviral study revealed that the formulated nanoparticles were found to have good viral activity.

**Yun Mo**[61] ***et al.,*** developed a lectin-conjugated isopropyl myristate (IPM)-incorporated PLGA nanoparticle system (NP) for the local delivery of paclitaxel to the lungs. Wheat germ agglutinin (WGA) was conjugated onto preformed IPM- and paclitaxel-loaded PLGA NPs by a two-step carbodiimide method. WIT-NP with mean diameter of 331 nm and zeta potential of _4.3 mV were prepared with yield of 66% and paclitaxel encapsulation efficiency of 61%. Particle size was expanded by surface conjugation with WGA, while zeta potential was reduced by the addition of IPM and WGA. In vitro paclitaxel release profile was not affected by WGA but initial drug release was enhanced by adding IPM into the formulation.

**L.X. Tiefenauer**[62] ***et al.,*** developed magnetite nanoparticles, coated by three different artificial polypeptides, conjugated to an antibody specific to the carcinoembryonic antigen (CEA). To protect the particles from fast blood elimination, the coats were modified by various sugars, polyethyleneglycol, albumin, and sialoproteins, respectively. The protective effect was determined by using a specific in vitro test and by analyzing the biodistrlbution of the nanoparticles in nude mice grafted with CEA-tumors. In particular, a prolongation of the blood circulation time has been expected, if a natural modifier is attached to the coated nanoparticles. The usefulness of nanoparticles as image contrast agents is probably limited by their microdistribution within the tumor tissue.

**Asuman Bozkir**[63] ***et al.,*** designed to optimize the formulation of 5-fluorouracil (5-FU) loaded poly D, L (lactide-co- glycolide) (PLGA) nanoparticles (5FU-NP) by a nanoprecipitation-solvent displacement technique. Optimized formulations have the particle

size ranging from 160 to 250 nm. Smallest nanoparticles (161 ± 1.22 nm) were obtained using Resomer PLGA 755 and pluronic F-68 with 10 ml acetone amount. Under these conditions the 5-FU entrapment percentage was maximum 78.30%, suggesting 5-FU might be entrapped and adsorbed on the nanoparticle surface. In vitro release of the formulations with maximum drug entrapment efficiency and minimum particle size, were also investigated by release kinetics. According to the determined coefficients, release data fit to Higuchi's diffusion kinetics. The in vitro release of 5FU-NP in phosphate buffered saline (PBS, pH 7.4) is suggested to be controlled by a combination of diffusion with slow and gradual erosion of the particles.

**C. Schwarz**[64] ***et al.,*** prepared solid lipid nanoparticles (SLN) of a quality acceptable for i.v. administration. Dynasan 112 and Compritol ATO 888 were used as lipid matrices for the SLN, stabilisers were Lipoid S 75 and poloxamer 188, respectively. Changes in particle size distribution during lyophilisation could be minimised by optimising the parameters of the lyophilisation process, i.e. freezing velocity and redispersion method. Lyophilised drug-free SLN could be reconstituted in a quality considered suitable for i.v. injection with regard to the size distribution. Loading with model drugs (tetracaine, etomidate) impairs the quality of reconstituted SLN. However, the lyophilisate quality is sufficient for formulations less critical to limited particle growth.

**P.K. Gupta**[65] ***et al.,*** described the methods of evaluating targeted drug delivery systems have been reviewed. It has been observed that in most instances the parameters used for the evaluation do not necessarily provide true quantitative differences between the selectivity of test and conventional delivery systems. It has been shown that the inadequate collection of data may lead to misinterpretation of the efficacy of drug delivery systems. Some mathematical relationships have been suggested and their usefulness substantiated with the aid of data describing the disposition of adriamycin administered to rats as a solution and via liposomes.

# AIM OF WORK

Targeted drug delivery system is designed to improve the risk ratio of any drug. It decreases the distribution in unwanted tissues and makes the drug more available in the required tissue.

The present study was aimed to develop glimepiride nanoparticle. Since glimepiride has poor solubility, cyclodextrin complexes are used to enhance the solubility. It results in prolonged action with controlled release manner.

Glimepiride is a sulphonylurea group used for the lowering of blood glucose level which belongs to third generation hypoglycemic drug with long duration of action. Currently available dosage form shows some side effects like dizziness, fast heartbeat, itching, tightness in the chest wheezing, bleeding gums, dark urine, headache, fever.

Thus the present investigation was carried out for the following objectives;

- Prolonged action
- Controlled release
- To achieve maximum bioavailability
- To reduce side effects
- To improve solubility
- To overcome therapeutic efficacy

## PLAN AND SCOPE OF WORK

Plan of the work involves the following process;

- Preformulation studies which involves the observation of physical and chemical data. The infrared spectrophotometry is used to identify the raw materials and compatibility studies between drug and the polymer used.
- Fabrication of glimepiride cyclodextrin complexes nanoparticles is done by kneading method.
- Fabrication of the nanoparticles in various ratios of the drug and the polymer.

Based on the result of the following parameters, the best formulation will be selected. The prepared nanoparticle is evaluated by the following chemical characteristics.

- Drug content
- Drug entrapment efficiency
- *In vitro* drug release studies
- Scanning electron microscope
- Zeta potential analysis
- Stability studies at different temperature
- Drug release kinetics studies

## INSTRUMENTS AND MATERIALS USED:

**Table 4: Instruments used**

| S.NO | EQUIPMENTS | SUPPLIERS |
|---|---|---|
| 1 | Rotary flash evaporator | Equitron, Mumbai. |
| 2 | Probe sonicator | Bandelin, Germany. |
| 3 | Single beam UV spectrophotometer | Shimadzu corporation, Japan. |
| 4 | Electronic balance | Shimadzu. |
| 5 | FTIR spectrophotometer | Perkin Elmer, Germany. |
| 6 | Double beam UV spectrophotometer | Perkin Elmer, Germany. |
| 7 | Vortex mixer | Remi motors Ltd, Mumbai. |
| 8 | Magnetic stirrer | Remi motors Ltd. |
| 9 | Stability chamber | Osword, Mumbai. |
| 10 | Ultra centrifugation | Remi motors Ltd, Mumbai. |
| 11 | pH meter | Elico Pvt Ltd, Chennai. |
| 12 | Hot air oven | Biochemicals, Mumbai. |

**Table 5: Materials used**

| S.NO | MATERIALS | SUPPLIER |
|---|---|---|
| 1. | Glimepiride | Yashica pharmaceutical Pvt Ltd, Thane. |

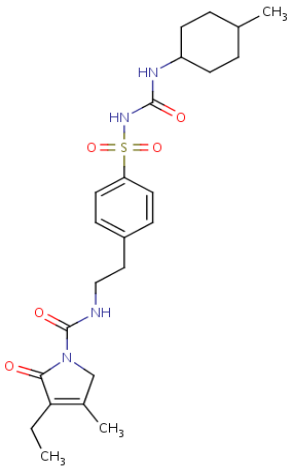
| | | |
|---|---|---|
| 2. | β- cyclodextrin | Bafna pharmaceuticals, Mumbai. |
| 3. | Poly ethylene glycol 6000 | Microlabs, Hosur. |
| 4. | Tween 80 | Microlabs, Hosur. |
| 5. | Acetone | Marck, India. |
| 6. | Potassium dihydrogen phosphate | S.D.Fine chemicals Ltd, Boisar. |
| 7. | Sodium chloride | S.D.Fine chemicals Ltd, Boisar. |
| 8. | Membrane filter | Gotting Ltd, West Germany. |
| 9. | Ethanol | Marck, India. |

## DRUG PROFILE:

### GLIMEPIRIDE:

1-[[p- [2-(3- ethyl-4-methyl-2-oxo-3-pyrroline1- carboxamido) ethyl] phenyl] sulphonyl]-3-(trans-4-methyl cyclohexyl) urea.

**Chemical structure:**



**Molecular formula:**

$C_{24}H_{34}N_4O_5S$

**Description:**

A white powder.

**Melting point:**

207 °C

**Solubility:**

Insoluble in water, soluble in acetone, dimethyl formamide.

**Pharmacology:**

In Type 2 diabetes, it is considered that the lowered insulin secretion and the lowered insulin sensitivity cause hyperglycemia. Glimepiride, a new sulfonylurea, has a blood-glucose lowering effect as strong as those of existing sulfonylureas, but only induces mild insulin secretion. The association and dissociation to the sulfonylurea receptor of glimepiride are

faster. Additionally, it is confirmed by basic studies that part of the glimepiride effect is attributable to improving insulin sensitivity. Glimepiride has already been used in more than 60 countries in the world. Glimepiride is expected to be a new efficient agent for the treatment of Type 2 diabetes.

**Mechanism of action:**

The mechanism of action of glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells. Glimepiride likely binds to ATP-sensitive potassium channel receptors on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. This increase in intracellular calcium ion concentration induces the secretion of insulin.

**Pharmacodynamics:**

Glimepiride is used with diet to lower blood glucose by increasing the secretion of insulin from pancreas and increasing the sensitivity of peripheral tissues to insulin.

**Pharmacokinetics:**

**Absorption:**

Studies with single oral doses of glimepiride in healthy subjects and with multiple oral doses in patients with type 2 diabetes showed peak drug concentrations (Cmax) 2 to 3 hours post-dose. When glimepiride was given with meals, the mean Cmax and AUC (area under the curve) were decreased by 8% and 9%, respectively.

Glimepiride does not accumulate in serum following multiple dosing. Clearance of glimepiride after oral administration does not change over the 1 mg to 8 mg dose range, indicating linear pharmacokinetics.

**Distribution:**

After intravenous dosing in healthy subjects, the volume of distribution (Vd) was 8.8 L (113 mL/kg), and the total body clearance (CL) was 47.8 mL/min. Protein binding was greater than 99.5%.

**Metabolism:**

Glimepiride is completely metabolized by oxidative biotransformation after either an intravenous or oral dose. The major metabolites are the cyclohexyl hydroxy methyl derivative (M1) and the carboxyl derivative (M2). Cytochrome P450 2C9 is involved in the biotransformation of glimepiride to M1. M1 is further metabolized to M2 by one or several cytosolic enzymes. M2 is inactive.

**Excretion:**

When C-glimepiride was given orally to 3 healthy male subjects, approximately 60% of the total radioactivity was recovered in the urine in 7 days. M1 and M2 accounted for 80-90% of the radioactivity recovered in the urine. The ratio of M1 to M2 in the urine was approximately 3:2 in two subjects and 4:1 in one subject. Approximately 40% of the total radioactivity was recovered in faeces. M1 and M2 accounted for approximately 70% (ratio of M1 to M2 was 1:3) of the radioactivity recovered in faeces. No parent drug was recovered from urine or faeces. After intravenous dosing in patients, no significant biliary excretion of glimepiride or its M1 metabolite was observed.

**Adverse effects:**

Side effects from taking glimepiride include gastrointestinal tract (GI) disturbances, occasional allergic reactions, and rarely blood production disorders including thrombocytopenia, leukopenia, and hemolytic anemia. In the initial weeks of treatment, the risk of hypoglycemia may be increased. Alcohol consumption and exposure to sunlight should be restricted because they can worsen side effects.

**Dosage:**

Glimepiride tablets USP 4mg once a day.

**Storage:**

Stored at 20° to 25°C (68° to 77°F).

## POLYMER PROFILE:

### β- CYCLODEXTRIN:

**Non- proprietary names:**

BP: betadex

USPNF: betadex

**Synonyms:**

Beta-cycloamyloses,beta-dextrin, cavamax W7 pharma, cyclohepta amylase, cycloheptaglucan, cyclomaltoheptose, kleptose.

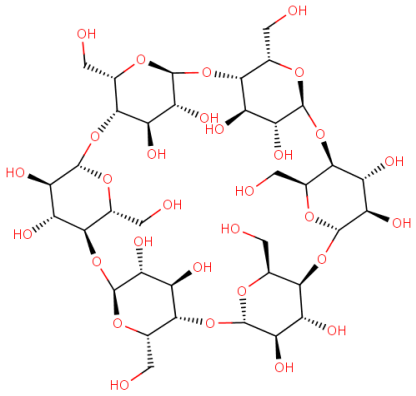
**Chemical name and CAS registry number:**

β- Cyclodextrin [7585-39-9]

**Empirical formula and molecular weight:**

β- Cyclodextrin $C_{42}H_{70}O_{35}$ =1135.

**Structural formula:**



Note: the structure of β- cyclodextrin (7 glucose units) is shown.

R', R'' = H for natural α, β, γ- cyclodextrins.

R', R'' = $CH_3$ for methyl cyclodextrins.

R', R'' = $CHOCH_3$ for 2- hydroxyethyl cyclodextrins.

R', R'' = $CH_2CHOHCH_3$ for 2- hydroxypropyl cyclodextrins.

**Functional category:**

Solubilising agents, stabilising agents.

**Applications in pharmaceutical formulations or technology:**

β- Cyclodextrin is the most commonly used cyclodextrin, although it is the least soluble. It is the least expensive cyclodextrin. It is commercially available from a number of sources and it is able to form inclusion complexes with a number of molecules of pharmaceutical interest. However, β- cyclodextrin is nephrotoxic and should not be used in parental formulations.

In parental formulation, cyclodextrin have been used to produce stable and soluble preparations of drugs that would otherwise have been formulated using a non- aqueous solvent. In eye drop formulation, cyclodextrin form water soluble complexes with lipophilic drugs such as corticosteroids. They have been shown to increase the water solubility of the drug to enhance the drug absorption into the eye, to improve the aqueous stability and to reduce local irritation.

Cyclodextrin have also been used in the formulation of solutions, suppositories and cosmetics.

**Description:**

Cyclodextrins occur as white, practically odourless, fine crystalline powder having a slightly sweet taste. Some cyclodextrin derivatives occur as amorphous powder.

**Typical properties:**

| | |
|---|---|
| Compressibility | _ 21.0 – 44.0% for β- cyclodextrin. |
| Density (bulk) | _ 0.523 g/$cm^3$. |
| Density (tapped) | _ 0.754 g/$cm^3$. |
| Melting point | _ 255 – 265$^0$C. |
| Moisture content | _ 13.0 – 15.0% w/w. |

Particle size distribution _ 7.0 – 45.0 μm.

**Physical characteristics:**

| **Characteristics** | **β- cyclodextrin** |
|---|---|
| Cavity diameter (Å) | - 6.0 - 6.5 |
| Height of torus (Å) | - 7.9 |
| Diameter of periphery (Å) | - 15.4 |
| Approximate volume of cavity (Å) | - 262 |
| Approximate cavity volume | |
| Per mol cyclodextrin (ml) | - 157 |
| Per gram cyclodextrin (ml) | - 0.14 |

**Solubility:**

β- Cyclodextrin is soluble in 1 in 200 parts of PEG, 1 in 50 parts of water at 20° C, practically insoluble in acetone, ethanol (95%) and methylene chloride.

**Surface tension:** (at 25° C)

β- CD: 71 dynes/cm.

**Stability and storage conditions:**

β- cyclodextrin and other cyclodextrins are stable in solid state if protected from high humidity. Cyclodextrins should be stored in a tightly sealed container in a cool, dry place.

**Incompatibilities:**

The activity of some anti-microbial preservatives in aqueous solution can be reduced in the presence of hydroxypropyl-β- CD.

**Safety:**

Cyclodextrins are starch derivatives and are mainly used in oral and parentral pharmaceutical formulation. They are used in topical and ophthalmic formulations.

**POLYEHYLENE GLYCOL 6000:**

**Pharmacy equivalent name:**

PEG6000

**Chemical name:**

Poly (oxy- 1, 2- ethanediyl), 2- hydroxy polyethylene glycol.

**Category:**

Pharmaceutical aid (ointment base, suppository base, tablet excipients).

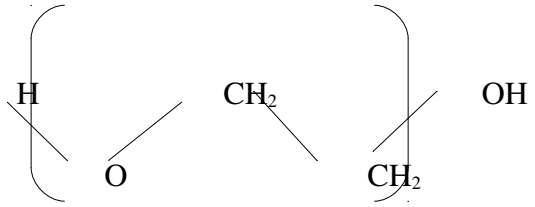
**Description:**

White free flowing powder or creamy white flasks. It is practically odourless and tasteless.

**Solubility:**

Freely soluble in water, alcohol and chloroform. Insoluble in ether.

**Structure:**




PEG6000 is a polymer of ethylene oxide and water, represented by the formula $H(OCH_2CH_2)nOH$, in which the average value of n lies between 158 and 204.

**Congealing range:**

Between 56 and 63.

**Average molecular weight:**

The average molecular weight is between 7000 and 9000.

**Viscocity:**

Between 470 centistrokes and 900 centistrokes, at 210$^{o}$F, expressed kinematics viscosity.

**Storage:**

Stored in tightly closed container.

**Uses:**

Generally PEG6000 is used in chewable tablets and tablet formulation that require water solubility. It is a non-reactive and can be used in pH sensitive (stable) drugs such as aspirin and vitamins. PEG solutions can be sprayed in aqueous or hydroalcoholic solvents into powders in twin- shell or double- cone blenders. However, they are not available in small particle size. Also used if food and food packaging. It may also be used in cosmetics and in ointments.

**TWEEN 80:**

**Synonym:**

Polysorbate 80, armotan PMO 20, Tween 80.

**Chemical name:**

Polyoxy ethylene 20 sorbitan mono oleate.

**Molecular weight:**

1310.

**Functional category:**

Emulsifying agent, non- ionic surfactant, solubilising agent, wetting, dispersing or suspending agent.

**Description:**

Polysorbates have a characteristic odour and a warm, somewhat bitter taste. The colour of polysorbate 80 is yellow, it is an oily liquid.

**Properties:**

Acidty/ alkalinity: pH=6.0 – 8.0 for a 5% w/v aqueous solution.

Solubility: soluble in ethanol and water, insoluble in mineral oil and vegetable oil.

**Incompatibilities:**

Discolouration and or precipitation occur with various substance especially phenols, tannins, tars and tarlike materials. The anti- microbial activity of paraben preservatives is reduced in presence of polysorbates.

## CONSTRUCTION OF STANDARD CURVE OF GLIMEPIRIDE:

### By UV spectroscopy method:

Glimepiride is estimated spectrophotometrically at 228nm and it obeys Beer-Lambert's law in the range of 1-10µg/ml.

### Determination of absorbance maximum ($\lambda_{max}$):

Glimepiride was dissolved in phosphate buffer saline pH 7.4. Solution with 10µg/ml concentration was prepared by suitable dilution. The solution was scanned in UV spectrophotometer at 200-400 nm using phosphate buffer saline pH 7.4 as blank. Absorbance maximum was determined at 228nm. The drug was later quantified by measuring the absorbance at 228nm in phosphate buffer saline pH 7.4.

### Preparation of pH 7.4 phosphate buffer saline:[66]

6.8gms of potassium dihydrogen orthophosphate and 1.6gms of sodium hydroxide were accurately weighed and transferred into 1000ml volumetric flask. The volume is made upto 1000ml with distilled water. The pH was adjusted if necessary.

### Preparation of stock solution:

Stock solution was prepared by dissolving 50mg of glimepiride in 50ml of the solvent medium, so as to get a solution of 1000µg/ml concentration (primary stock solution). From the primary stock solution, 1ml was taken in 100ml standard flask and it is diluted to 100ml with the solvent medium (secondary stock solution) to get a concentration of 10µg/ml.

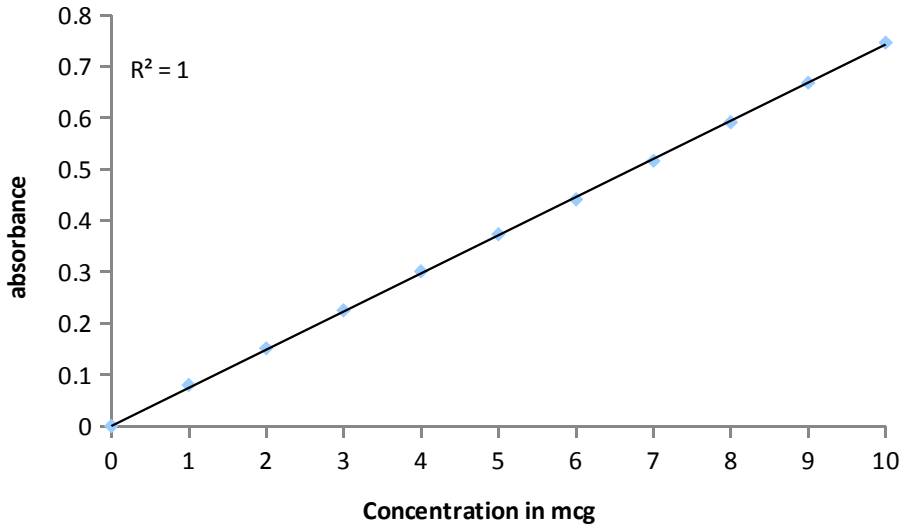
### Preparation of standard solution:

From the secondary stock solution, aliquots ranging from 1-10 ml was taken and made upto 10ml with the solvent medium to get the final concentration ranging from 1-10µg/ml. Absorbance of the solution was measured at 228nm UV spectrophotometrically against drug free phosphate buffer pH 7.4 media as blank.

**Table 6: Calibration curve of glimepiride at 228nm:**

| S.No | Concentration (µg/ml) | Absorbance at 228nm |
|---|---|---|
| 1 | 1 | 0.080 |
| 2 | 2 | 0.151 |
| 3 | 3 | 0.225 |
| 4 | 4 | 0.301 |
| 5 | 5 | 0.373 |
| 6 | 6 | 0.441 |
| 7 | 7 | 0.516 |
| 8 | 8 | 0.591 |
| 9 | 9 | 0.668 |
| 10 | 10 | 0.746 |

**Fig 2: Standard curve of glimepiride:**




## METHOD OF PREPARATION OF GLIMEPIRIDE- CYCLODEXTRIN COMPLEXES:

### Kneading method:

The glimepiride-cyclodextrin complexes were prepared by kneading method. 10mg of the drug, 4mg of β- cyclodextrin and 20mg poly ethylene glycol, 2mg of tween-80were taken. The mixture was heated and 20ml of acetone was added to it. This was then sonicated using probe sonicator set at 60W of energy output for 90 secs. This was further evaporated by flash rotatory evaporator for 60mins.

**Table 7: Various compositions of formulations:**

| S.No | Formulation code | Drug (mg) | β- CD (mg) | PEG (mg) | Tween 80 mg | ratio |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | CD1 | 4 | 4 | 20 | 2 | 1:1:5:2 |
| 2 | CD2 | 4 | 8 | 20 | 2 | 1:2:5:2 |
| 3 | CD3 | 4 | 12 | 20 | 2 | 1:3:5:2 |
| 4 | CD4 | 4 | 16 | 20 | 2 | 1:4:5:2 |
| 5 | CD5 | 4 | 20 | 20 | 2 | 1:5:5:2 |
| 6 | CD6 | 4 | 24 | 20 | 2 | 1:6:5:2 |
| 7 | CD7 | 4 | 28 | 20 | 2 | 1:7:5:2 |
| 8 | CD8 | 4 | 32 | 20 | 2 | 1:8:5:2 |
| 9 | CD9 | 4 | 36 | 20 | 2 | 1:9:5:2 |
| 10 | CD10 | 4 | 40 | 20 | 2 | 1:10:5:2 |

**EVALUATION OF NANOPARTICLES: [67]**

**Drug entrapment study:**

The entrapment efficiency study was determined by free drug content in the supernatant which is obtained after centrifugation at 15000rpm for 20mins at $0^0$ using ultra centrifuge. The absorbance was measured by UV spectrophotometer at 228nm.

**IN VITRO DRUG RELEASE STUDIES: [68, 69.]**

**By UV spectrophotometric method: [70]**

The *in vitro* drug release study was carried out using the diffusion membrane technique. The nanoparticle preparation was placed in a dialysis membrane and it is kept in a beaker containing 100ml of diffusion medium (phosphate buffer pH 7.4). The medium was maintained at $37^0$ C under magnetic stirring at constant speed. 1ml of the sample was taken from the diffusion medium at a fixed time interval. 1ml of fresh medium was replaced. This process was carried out for 24 hours. The sample was measured at 228nm using UV spectrophotometer. The percentage of drug released at various time intervals was calculated from calibration graph.

**Morphology of nanoparticles by simple microscopy:**

The optimized formulation was morphologically characterized by microscopy. A small amount of sample was placed in a glass slide and investigated in microscopy.

**Scanning electron microscopy:** [71]

The formulation was morphologically characterized using scanning electron microscopy (SEM). For SEM analysis, the sample was mounted in a scotsch double adhesive tape. The sample was analysed in hitachi scanning electron microscope operated at 15 kv and photograph was taken.

**Surface charge (zeta potential) determination:** [72]

Zeta potential is an important parameter to evaluate an optimum condition for stability of colloidal or dispersed systems. The prepared nanoparticles were characterized by using zeta potential analyser (Malvern Zeta seizer). Zeta potential creates an electrical barrier. It is very important for drug stability. The effect of β- cyclodextrin complexes on the surface of the nanoparticles was studied.

**pH and physical appearance:**

The pH of the formulation was measured using pH meter. It plays a role in the process of stability and formulation activity. The physical appearance of the formulation like colour and suspended foreign particulate were to be examined.

**Stability studies of nanoparticles:**

The stability studies of nanoparticles involve observing the formulation at $45^0$C/70% RH which constitutes accelerated condition at $4^0$C on refrigerator and room temperature. At both the temperature the formulation was kept for 3 months. Small amount of the sample was withdrawn at periodic intervals for performing the following tests.

a) Physical appearance
b) *In vitro* drug release (dissolution)
c) pH of the solution
d) Percentage of drug entrapment.

# RESULTS AND DISCUSSION

## DEVELOPMENT OF GLIMEPIRIDE-CYCLODEXTRIN COMPLEXES:

The glimepiride cyclodextrin complexes were prepared by kneading method. 4mg of glimepiride, 4mg β- cyclodextrin, 20mg poly ethylene glycol, 2mg Tween 80 were taken. The mixture was heated and 20ml of acetone was added to it. This was then sonicated using Probe sonicator set at 60W of energy output for 90secs. This was further evaporated by flash rotatory evaporator for 60mins.

Formulations with different ratios of polymer were prepared. Several physicochemical characteristics of nanoparticles like morphology, particle size determination, drug release profile were investigated. Stability of the formulation at various temperatures was evaluated.

## DRUG AND POLYMER COMPATIBILITY STUDIES BY FTIR: [73, 74, 75.]

Infrared spectroscopy by potassium bromide pellet method was carried out on pure substance (glimepiride and β- cyclodextrin complexes) seperately and their physical mixtures. They are compressed under 15 tonnes pressure in a hydraulic press to form a transparent pellet. Then the pellet was scanned from 4000-400 $cm^{-1}$ in a spectrophotometer.

To determine any possible molecular interactions between the drug and the polymer, the spectrum of physical mixture was compared with the original spectra. FTIR analysis measures the selective absorbance of light by the viberation modes of specific chemical bonds in the sample.

They are compressed under 15 tonnes pressure in a hydraulic press to form a transparent pellet. The pellet was scanned from 4000-400 $cm^{-1}$ in a spectrophotometer.

Spectrum Pathname: C:\PEL_DATA\SPECTRA\GLIMEPIRIDE.002

GLIMEPIRIDE.pk

GLIMEPIRIDE.002 3601 4000.00 400.00 2.33 57.85 4.00 %T 4 1.00

REF 4000 57.85 2000 55.20 600
3932.32 55.28 3905.86 52.05 3872.24 53.16 3856.22 51.64 3841.13 51.71
3823.31 52.07 3805.48 51.88 3753.57 49.80 3737.31 53.66 3713.59 52.55
3693.03 51.22 3677.44 49.98 3653.60 48.62 3632.58 49.66 3593.64 50.33
3570.03 48.06 3530.40 47.40 3370.34 21.14 3289.88 29.62 3135.69 40.76
2932.06 20.28 2861.16 28.16 2372.99 51.38 2343.57 53.32 2237.67 54.18

Spectrum Pathname: C:\PEL_DATA\SPECTRA\ GLIMEPIRIDE CYCLODEXTRIN COMPLEXES.002

GLIMEPIRIDE CYCLODEXTRIN COMPLEXES.pk

GLIMEPIRIDE CYCLODEXTRIN COMPLEXES.002 3601 4000.00 400.00 41.38 56.11 4.00 %T 4 1.00

REF 4000 56.11 2000 54.11 600
3905.18 52.98 3805.20 52.50 3753.78 50.33 3679.12 50.68 3652.31 49.00
3427.05 41.38 2919.86 46.59 2369.88 50.32 1702.15 51.25 1655.10 49.82
1561.54 50.45 1459.14 51.51 1351.65 51.29 1099.71 44.58 950.19 52.76
839.48 53.97 586.29 53.20

Table 8: IR spectra data for pure drug glimepiride:

| Frequency $cm^{-1}$ | Groups assigned |
|---|---|
| 3370 | N-H stretching |
| 3135 | Aromatic C-H stretching |
| 2932 | C-H stretching |
| 1700 | C=O stretching |
| 1115 | C-N stretching |

Table 9: IR spectra data for β- cyclodextrin complexes:

| Frequency $cm^{-1}$ | Groups assigned |
|---|---|
| 2919 | Aliphatic C-H stretching |
| 1702 | C=O stretching |
| 1099 | C-N stretching |

In FTIR spectra the peaks of physical mixture was compared with the original spectra. Same peaks were observed, there is no possible molecular interactions between the drug and the polymer.

**ENTRAPMENT EFFICIENCY OF NANOPARTICLES:** [76.]

The entrapment efficiency of glimepiride loaded nanoparticles was prepared by kneading method. The formulation CD1 (GMP 4mg, β- CD 4mg, PEG 20mg, tween 80 2mg) showed entrapment efficiency value of 49%. This is due to the repulsive force between drug and the polymer.

Increase the cyclodextrin concentration in formulation CD2, (GMP 4mg, β- CD 8mg, PEG 20mg, tween 80 2mg) was used. The entrapment value was 56%. The cyclodextrin concentration was increased to 12, 16 and 20mg in the formulation CD3, CD4, CD5 and showed the entrapment efficiency value of 60%, 61% and 68%.

There was a steady increased in the entrapment efficiency from 72%, 73%, 83%, 85% in the formulation CD6, CD7, CD8 and CD9.

The concentration of β- CD was further increased to 40mg in CD10 formulation. PEG and tween 80 were kept constant in all the formulations. The E.E value showed 94%. This is due to the high repulsive force between drug and the polymer.

Table 10: Entrapment efficiency formulations with drug and polymer:

| S.No | Formulation code | Drug(mg) | β-CD(mg) | PEG(mg) | Tween80 (mg) | E.E (%) |
|---|---|---|---|---|---|---|
| 1 | CD1 | 4 | 4 | 20 | 2 | 49±0.14 |
| 2 | CD2 | 4 | 8 | 20 | 2 | 56±0.11 |
| 3 | CD3 | 4 | 12 | 20 | 2 | 60±0.08 |
| 4 | CD4 | 4 | 16 | 20 | 2 | 61±0.12 |
| 5 | CD5 | 4 | 20 | 20 | 2 | 68±0.09 |
| 6 | CD6 | 4 | 24 | 20 | 2 | 72±0.17 |
| 7 | CD7 | 4 | 28 | 20 | 2 | 73±0.12 |
| 8 | CD8 | 4 | 32 | 20 | 2 | 83±0.17 |
| 9 | CD9 | 4 | 36 | 20 | 2 | 85±0.08 |
| 10 | CD10 | 4 | 40 | 20 | 2 | 94±0.05 |

**INVITRO DRUG RELEASE PROFILE ON NANOPARTICLES:**

- The in vitro drug release of glimepiride nanoparticles was carried out by membrane diffusion method. It was carried out for 24hours.
- In all the formulations, pure drug glimepiride 4mg was taken. PEG 20mg and tween 80 2mg were taken constant in all the ten formulations. The concentration of β- cyclodextrin was gradually increased in each formulation to achieve the maximum release rate.
- In formulation CD1, β-CD 4mg was used to control the release of Glimepiride. The release at the end oh 24 hours was not found to be 63.1% only. It is not acceptable criteria of USP limit.
- Hence, in formulation CD2, β-CD concentration was increased to 3mg. The release rate at the end of 24 hours was found to be 65.6 %.
- To increase the release rate further, β-CD concentration was increased to12, 16,20mg in the formulations CD3, CD4, CD5 respectively. The release rate was increased to 78.2%, 80.7% and 83.0% respectively.
- Then in formulations CD6, CD7, CD8 and CD9. The concentration of β – cyclodextrin was further more increased to 24, 28, 32 and 36mg. Then the development in the release rate was observed with consequent increase in the release rate as 85.8%, 88.3%, 90.8% and 93.4%
- For further increase in the release rate, the concentration of β-CD was increased to 40mg in the formulation CD10.The release rate was found to be maximum. The release rate was 95%.
- Hence CD10 in the optimized formulation among the rest and formulation CD10 was chosen for further studies.

**Table 11: In vitro drug release for CD1:**

| Time (hrs) | Amount of drug release (mg) | Cumulative amount of drug release | Cumulative % drug release |
|---|---|---|---|
| 1 | 0.1 | 02.50 | 02.50 |
| 2 | 0.1 | 02.50 | 02.50 |
| 3 | 0.3 | 07.51 | 07.55 |
| 4 | 0.4 | 10.07 | 10.07 |
| 5 | 0.6 | 15.04 | 15.12 |
| 6 | 0.7 | 17.50 | 17.65 |
| 7 | 0.9 | 22.51 | 22.70 |
| 8 | 1.0 | 25.03 | 25.22 |
| 9 | 1.0 | 25.03 | 25.22 |
| 10 | 1.2 | 30.00 | 30.27 |
| 11 | 1.3 | 32.58 | 32.80 |
| 12 | 1.3 | 32.58 | 32.80 |
| 13 | 1.5 | 37.51 | 37.85 |
| 14 | 1.7 | 42.56 | 42.90 |
| 15 | 1.8 | 45.00 | 45.42 |
| 16 | 1.9 | 47.56 | 47.95 |
| 17 | 2.0 | 50.07 | 50.47 |
| 18 | 2.1 | 52.51 | 53.00 |
| 19 | 2.1 | 52.51 | 53.00 |
| 20 | 2.2 | 55.09 | 55.52 |
| 21 | 2.3 | 57.55 | 58.05 |
| 22 | 2.4 | 60.01 | 60.57 |
| 23 | 2.4 | 60.60 | 60.57 |
| 24 | 2.5 | 62.59 | 63.10 |

**Fig 3: In vitro drug release for formulation CD1:**

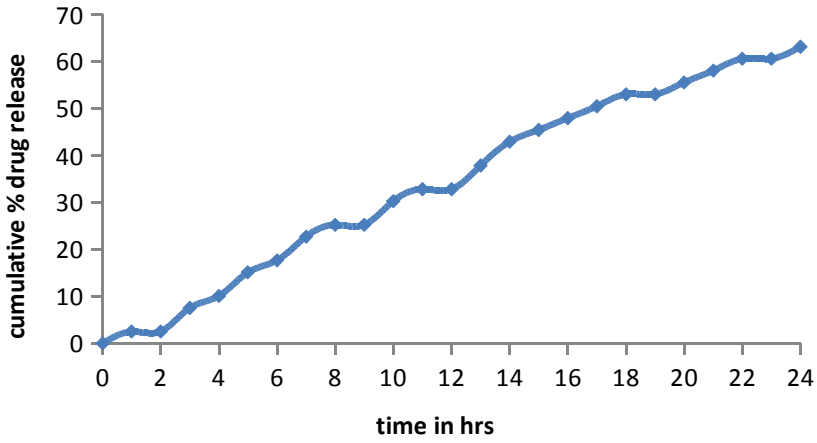



**Table 12: In vitro drug release for CD2:**

| Time (hrs) | Amount of drug release (mg) | Cumulative amount of drug release | Cumulative % drug release |
|---|---|---|---|
| 1 | 0.1 | 02.50 | 02.50 |
| 2 | 0.3 | 07.55 | 07.55 |
| 3 | 0.4 | 10.05 | 10.07 |
| 4 | 0.5 | 12.55 | 12.68 |
| 5 | 0.6 | 15.05 | 15.12 |
| 6 | 0.8 | 20.51 | 20.17 |
| 7 | 0.9 | 22.59 | 22.78 |
| 8 | 1.0 | 25.08 | 25.22 |
| 9 | 1.0 | 25.08 | 25.22 |
| 10 | 1.2 | 30.81 | 30.27 |
| 11 | 1.3 | 32.55 | 32.81 |
| 12 | 1.5 | 37.51 | 37.85 |
| 13 | 1.6 | 40.05 | 40.37 |
| 14 | 1.7 | 42.59 | 42.98 |
| 15 | 1.9 | 47.55 | 47.95 |
| 16 | 2.0 | 50.52 | 50.47 |
| 17 | 2.0 | 50.52 | 50.47 |
| 18 | 2.1 | 52.58 | 53.29 |
| 19 | 2.2 | 55.04 | 55.52 |
| 20 | 2.3 | 57.54 | 58.05 |
| 21 | 2.3 | 57.54 | 58.05 |
| 22 | 2.4 | 60.81 | 60.57 |
| 23 | 2.5 | 62.51 | 63.18 |
| 24 | 2.6 | 65.18 | 65.62 |

**Fig 4: In vitro drug release for formulation CD2:**

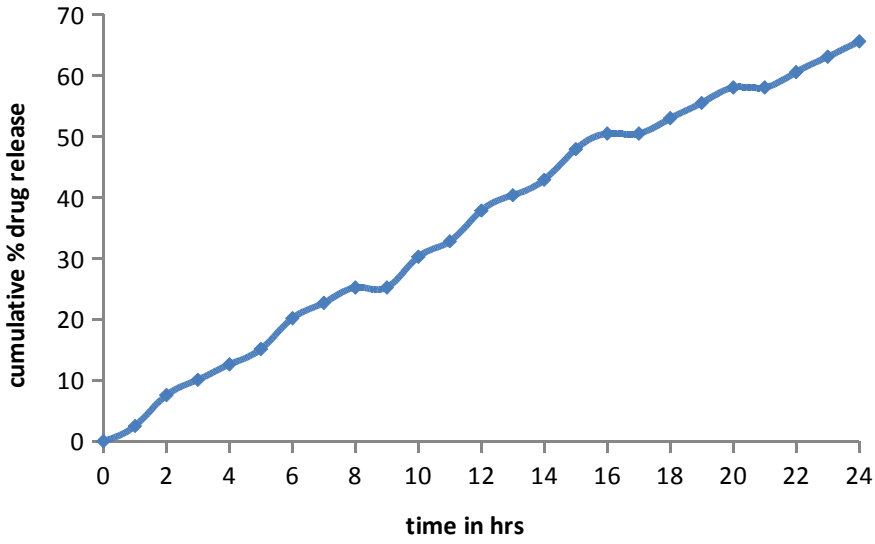


**Table 13: In vitro drug release for CD3:**

| Time (hrs) | Amount of drug release (mg) | Cumulative amount of drug release | Cumulative % drug release |
|---|---|---|---|
| 1 | 0.2 | 05.59 | 05.02 |
| 2 | 0.3 | 07.50 | 07.55 |
| 3 | 0.5 | 12.59 | 12.69 |
| 4 | 0.7 | 17.54 | 17.65 |
| 5 | 0.8 | 20.08 | 20.17 |
| 6 | 0.9 | 22.56 | 22.78 |
| 7 | 1.0 | 25.84 | 25.22 |
| 8 | 1.1 | 27.56 | 27.75 |
| 9 | 1.1 | 27.56 | 27.75 |
| 10 | 1.2 | 30.80 | 30.27 |
| 11 | 1.5 | 37.55 | 37.85 |
| 12 | 1.6 | 40.48 | 40.37 |
| 13 | 1.7 | 42.59 | 42.95 |
| 14 | 1.9 | 47.52 | 47.95 |
| 15 | 2.2 | 55.08 | 55.52 |
| 16 | 2.3 | 57.55 | 58.05 |
| 17 | 2.4 | 60.51 | 60.57 |
| 18 | 2.6 | 65.81 | 65.62 |
| 19 | 2.8 | 70.08 | 70.67 |
| 20 | 2.9 | 72.58 | 72.57 |
| 21 | 3.0 | 75.08 | 75.72 |
| 22 | 3.0 | 75.08 | 75.72 |
| 23 | 3.1 | 77.55 | 78.25 |
| 24 | 3.1 | 77.55 | 78.25 |

**Fig 5: In vitro drug release for formulation CD3:**

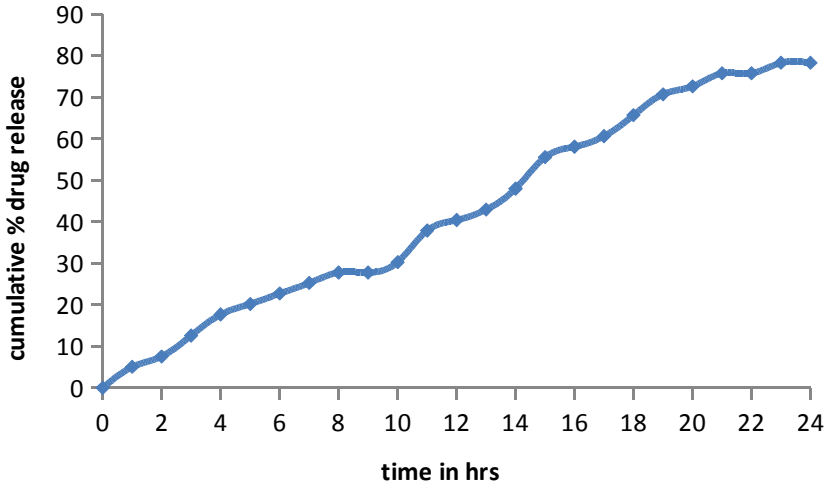


**Table 14: In vitro drug release for CD4:**

| Time (hrs) | Amount of drug release (mg) | Cumulative amount of drug release | Cumulative % drug release |
|---|---|---|---|
| 1 | 0.2 | 05.82 | 05.02 |
| 2 | 0.4 | 10.08 | 10.07 |
| 3 | 0.5 | 12.55 | 12.65 |
| 4 | 0.6 | 15.54 | 15.12 |
| 5 | 0.7 | 17.54 | 17.65 |
| 6 | 0.9 | 22.55 | 22.79 |
| 7 | 1.0 | 25.15 | 25.22 |
| 8 | 1.0 | 25.15 | 25.22 |
| 9 | 1.1 | 27.58 | 27.75 |
| 10 | 1.3 | 32.58 | 32.85 |
| 11 | 1.4 | 35.08 | 35.32 |
| 12 | 1.6 | 40.08 | 40.37 |
| 13 | 1.8 | 45.84 | 45.42 |
| 14 | 1.9 | 47.56 | 47.95 |
| 15 | 1.9 | 47.56 | 47.95 |
| 16 | 2.0 | 50.05 | 50.47 |
| 17 | 2.1 | 52.52 | 53.08 |
| 18 | 2.2 | 55.49 | 55.52 |
| 19 | 2.4 | 60.45 | 60.57 |
| 20 | 2.6 | 65.95 | 65.62 |
| 21 | 2.8 | 70.58 | 70.67 |
| 22 | 2.9 | 72.55 | 72.57 |
| 23 | 3.0 | 75.27 | 75.72 |
| 24 | 3.2 | 80.08 | 80.77 |

**Fig 6: In vitro drug release for formulation CD4:**

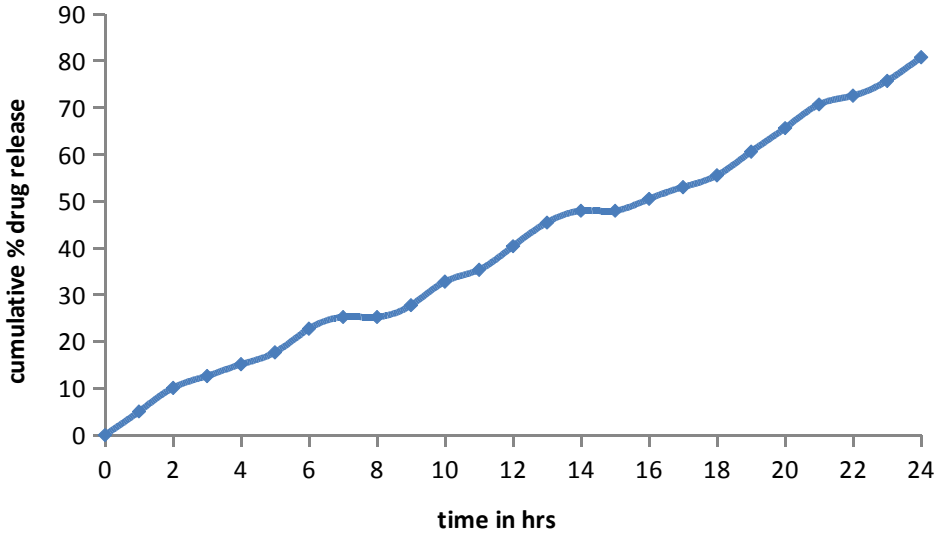



**Table 15: In vitro drug release for CD5:**

| Time (hrs) | Amount of drug release (mg) | Cumulative amount of drug release | Cumulative % drug release |
|---|---|---|---|
| 1 | 0.3 | 07.58 | 07.55 |
| 2 | 0.5 | 12.55 | 12.65 |
| 3 | 0.7 | 17.50 | 17.65 |
| 4 | 0.8 | 20.81 | 20.17 |
| 5 | 0.9 | 22.55 | 22.75 |
| 6 | 1.0 | 27.58 | 27.75 |
| 7 | 1.2 | 30.82 | 30.27 |
| 8 | 1.4 | 35.81 | 35.32 |
| 9 | 1.5 | 37.50 | 37.85 |
| 10 | 1.7 | 42.55 | 42.95 |
| 11 | 1.8 | 45.08 | 45.42 |
| 12 | 2.0 | 50.28 | 50.47 |
| 13 | 2.0 | 50.28 | 50.47 |
| 14 | 2.2 | 55.08 | 55.52 |
| 15 | 2.4 | 60.48 | 60.57 |
| 16 | 2.5 | 62.55 | 63.12 |
| 17 | 2.5 | 62.55 | 63.12 |
| 18 | 2.6 | 65.29 | 65.62 |
| 19 | 2.8 | 70.58 | 70.67 |
| 20 | 3.0 | 75.18 | 75.72 |
| 21 | 3.1 | 77.54 | 78.25 |
| 22 | 3.2 | 80.18 | 80.77 |
| 23 | 3.3 | 82.55 | 83.38 |
| 24 | 3.3 | 82.55 | 83.38 |

**Fig 7: In vitro drug release for formulation CD5:**

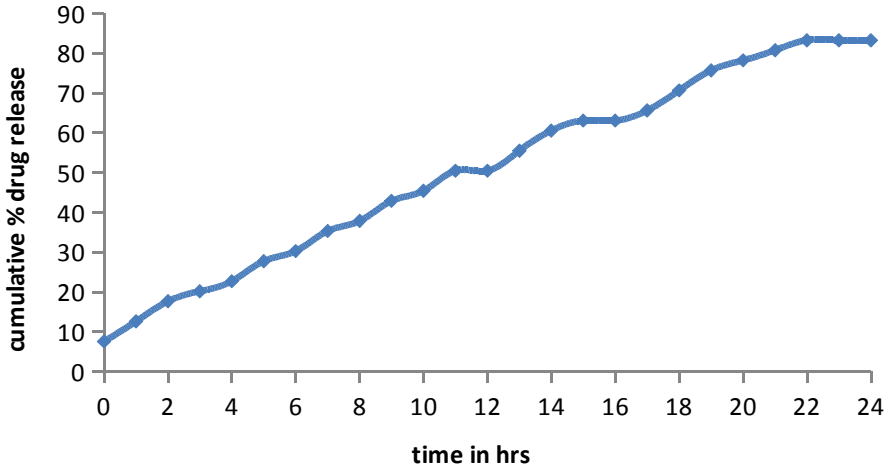



**Table 16: In vitro drug release for CD6:**

| Time (hrs) | Amount of drug release (mg) | Cumulative amount of drug release | Cumulative % drug release |
|---|---|---|---|
| 1 | 0.3 | 07.51 | 07.55 |
| 2 | 0.4 | 10.08 | 10.07 |
| 3 | 0.6 | 15.18 | 15.12 |
| 4 | 0.8 | 20.18 | 20.17 |
| 5 | 0.9 | 22.58 | 22.75 |
| 6 | 1.0 | 25.04 | 25.22 |
| 7 | 1.1 | 27.54 | 27.75 |
| 8 | 1.2 | 30.09 | 30.27 |
| 9 | 1.4 | 35.04 | 35.32 |
| 10 | 1.7 | 42.51 | 42.94 |
| 11 | 1.8 | 45.17 | 45.42 |
| 12 | 1.9 | 47.54 | 47.95 |
| 13 | 2.0 | 50.45 | 50.47 |
| 14 | 2.1 | 52.51 | 53.20 |
| 15 | 2.3 | 57.54 | 58.05 |
| 16 | 2.4 | 60.18 | 60.57 |
| 17 | 2.5 | 62.57 | 63.15 |
| 18 | 2.7 | 67.57 | 68.15 |
| 19 | 2.9 | 72.51 | 72.57 |
| 20 | 3.0 | 75.18 | 75.72 |
| 21 | 3.0 | 75.18 | 75.72 |
| 22 | 3.1 | 77.54 | 78.25 |
| 23 | 3.3 | 82.53 | 83.35 |
| 24 | 3.4 | 85.08 | 85.82 |

**Fig 8: In vitro drug release for formulation CD6:**

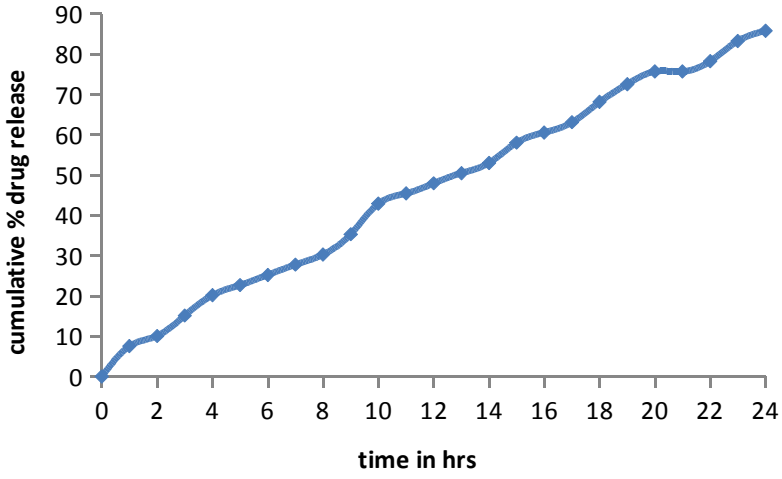



**Table 17: In vitro drug release for CD7:**

| Time (hrs) | Amount of drug release (mg) | Cumulative amount of drug release | Cumulative % drug release |
|---|---|---|---|
| 1 | 0.4 | 10.28 | 10.07 |
| 2 | 0.5 | 12.58 | 12.6 |
| 3 | 0.6 | 15.08 | 15.12 |
| 4 | 0.8 | 20.58 | 22.75 |
| 5 | 0.9 | 22.58 | 22.75 |
| 6 | 1.0 | 25.85 | 25.22 |
| 7 | 1.0 | 25.08 | 25.22 |
| 8 | 1.1 | 27.54 | 27.75 |
| 9 | 1.2 | 30.08 | 30.27 |
| 10 | 1.4 | 35.81 | 35.32 |
| 11 | 1.6 | 40.18 | 40.37 |
| 12 | 1.7 | 42.54 | 42.91 |
| 13 | 1.8 | 45.18 | 45.42 |
| 14 | 1.9 | 47.58 | 47.95 |
| 15 | 2.2 | 55.14 | 55.52 |
| 16 | 2.4 | 60.18 | 60.57 |
| 17 | 2.6 | 65.18 | 65.62 |
| 18 | 2.7 | 67.54 | 68.15 |
| 19 | 2.9 | 72.53 | 72.57 |
| 20 | 3.0 | 75.18 | 75.72 |
| 21 | 3.1 | 77.58 | 78.25 |
| 22 | 3.3 | 82.57 | 83.38 |
| 23 | 3.4 | 85.08 | 85.82 |
| 24 | 3.5 | 87.58 | 88.35 |

**Fig 9: In vitro drug release for formulation CD7:**

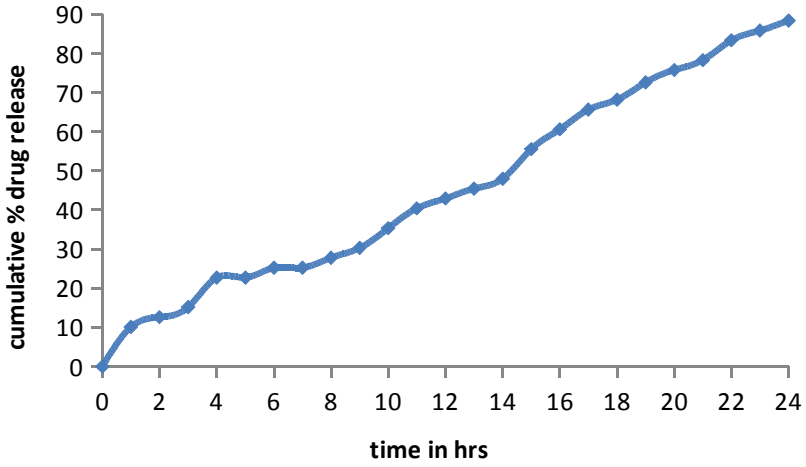



**Table 18: In vitro drug release for CD8:**

| Time (hrs) | Amount of drug release (mg) | Cumulative amount of drug release | Cumulative % drug release |
|---|---|---|---|
| 1 | 0.4 | 10.07 | 10.07 |
| 2 | 0.6 | 15.18 | 15.12 |
| 3 | 0.7 | 17.54 | 17.65 |
| 4 | 0.8 | 20.08 | 20.17 |
| 5 | 1.0 | 25.18 | 25.22 |
| 6 | 1.1 | 27.54 | 27.75 |
| 7 | 1.3 | 32.57 | 32.88 |
| 8 | 1.4 | 35.48 | 35.32 |
| 9 | 1.6 | 40.18 | 40.37 |
| 10 | 1.7 | 42.57 | 42.99 |
| 11 | 1.8 | 45.45 | 45.42 |
| 12 | 2.0 | 50.84 | 50.47 |
| 13 | 2.3 | 57.57 | 58.05 |
| 14 | 2.5 | 62.54 | 63.14 |
| 15 | 2.6 | 65.04 | 65.62 |
| 16 | 2.7 | 67.55 | 68.15 |
| 17 | 2.9 | 72.55 | 72.57 |
| 18 | 3.0 | 75.01 | 75.72 |
| 19 | 3.0 | 75.01 | 75.72 |
| 20 | 3.1 | 77.55 | 78.25 |
| 21 | 3.3 | 82.54 | 83.38 |
| 22 | 3.4 | 85.07 | 85.82 |
| 23 | 3.5 | 87.55 | 88.35 |
| 24 | 3.6 | 90.18 | 90.87 |

**Fig 10: In vitro drug release for formulation CD8:**

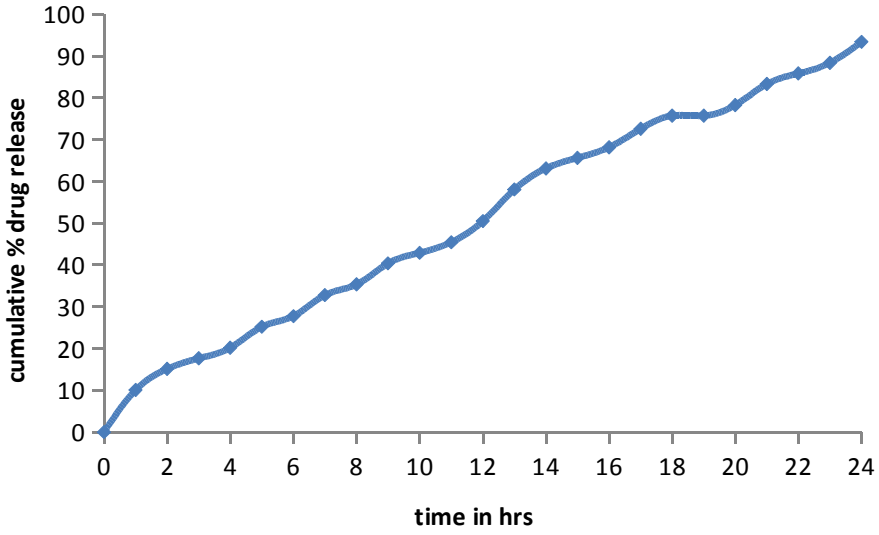



**Table 19: In vitro drug release for CD9:**

| Time (hrs) | Amount of drug release (mg) | Cumulative amount of drug release | Cumulative % drug release |
|---|---|---|---|
| 1 | 0.5 | 12.55 | 12.6 |
| 2 | 0.7 | 17.52 | 17.65 |
| 3 | 0.9 | 22.58 | 22.78 |
| 4 | 1.3 | 32.59 | 32.81 |
| 5 | 1.5 | 37.54 | 37.85 |
| 6 | 1.6 | 40.28 | 40.37 |
| 7 | 1.8 | 45.81 | 45.42 |
| 8 | 1.9 | 47.58 | 47.95 |
| 9 | 2.0 | 50.08 | 50.47 |
| 10 | 2.2 | 55.48 | 55.52 |
| 11 | 2.2 | 55.18 | 55.52 |
| 12 | 2.3 | 57.54 | 58.05 |
| 13 | 2.4 | 60.08 | 60.57 |
| 14 | 2.6 | 65.07 | 65.62 |
| 15 | 2.6 | 65.07 | 65.62 |
| 16 | 2.7 | 67.57 | 68.15 |
| 17 | 2.9 | 72.58 | 72.57 |
| 18 | 3.0 | 75.08 | 75.72 |
| 19 | 3.1 | 77.54 | 78.25 |
| 20 | 3.2 | 80.15 | 80.77 |
| 21 | 3.4 | 85.18 | 85.82 |
| 22 | 3.6 | 90.17 | 90.87 |
| 23 | 3.6 | 90.77 | 90.87 |
| 24 | 3.7 | 92.54 | 93.41 |

**Fig 11: In vitro drug release for formulation CD9:**

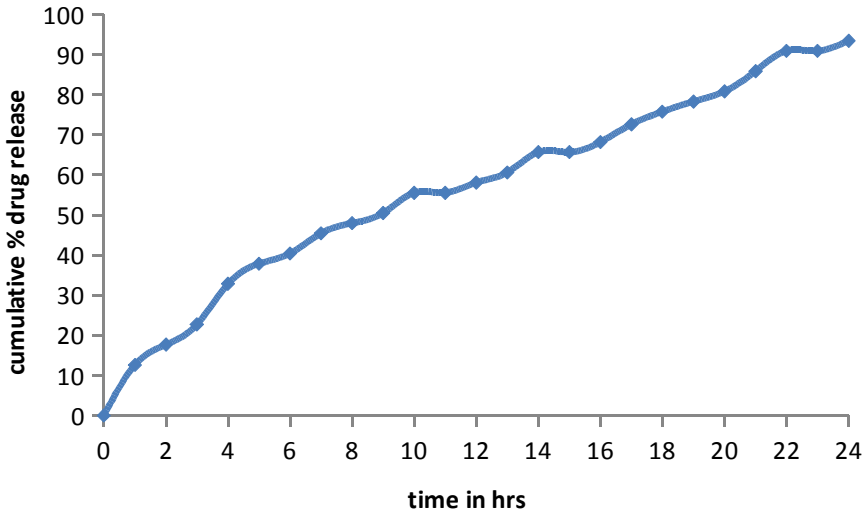



**Table 20: In vitro drug release for CD10:**

| Time (hrs) | Amount of drug release (mg) | % of drug release | Cumulative % drug release |
|---|---|---|---|
| 1 | 0.5 | 12.55 | 12.6 |
| 2 | 0.6 | 15.18 | 15.12 |
| 3 | 0.8 | 20.18 | 20.17 |
| 4 | 0.9 | 22.54 | 22.75 |
| 5 | 1.0 | 25.87 | 25.22 |
| 6 | 1.2 | 30.17 | 30.27 |
| 7 | 1.4 | 35.17 | 35.32 |
| 8 | 1.5 | 37.57 | 37.85 |
| 9 | 1.7 | 42.54 | 42.55 |
| 10 | 1.9 | 47.54 | 47.51 |
| 11 | 2.1 | 52.57 | 52.51 |
| 12 | 2.2 | 55.87 | 55.48 |
| 13 | 2.5 | 62.51 | 62.56 |
| 14 | 2.6 | 65.18 | 65.41 |
| 15 | 2.8 | 70.47 | 70.47 |
| 16 | 2.8 | 70.47 | 70.47 |
| 17 | 3.0 | 75.18 | 75.48 |
| 18 | 3.2 | 80.18 | 80.18 |
| 19 | 3.2 | 80.18 | 80.18 |
| 20 | 3.3 | 82.54 | 82.59 |
| 21 | 3.5 | 87.54 | 87.55 |
| 22 | 3.6 | 90.18 | 90.18 |
| 23 | 3.7 | 92.55 | 92.58 |
| 24 | 3.8 | 95.12 | 95.18 |

**Fig 12: In vitro drug release for formulation CD10:**

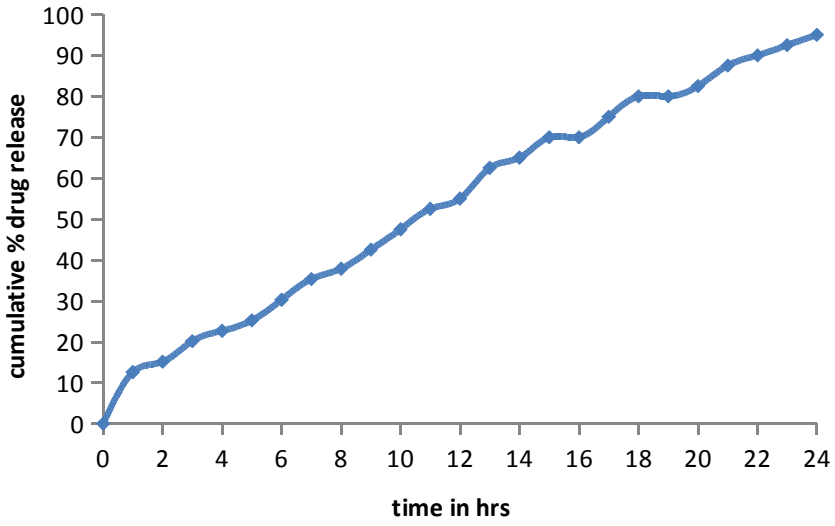
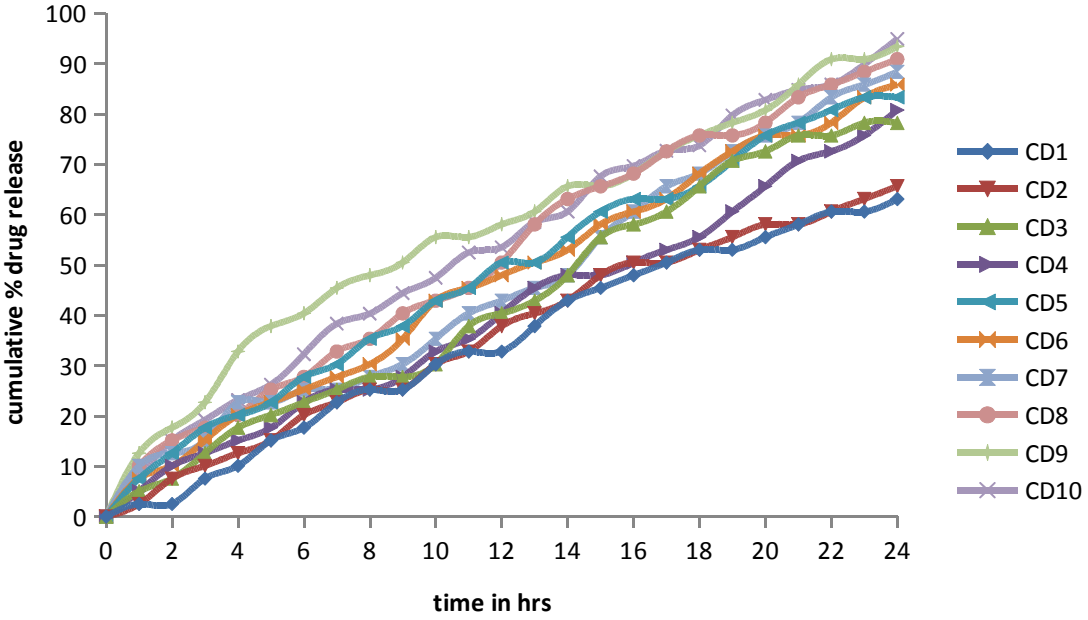



**Fig 13: Summarized in vitro drug release of nanoparticles formulation (CD1-CD10)**



**MORPHOLOGY OF NANOPARTICLES:**

The characteristics of CD10 formulation particle size was studied by simple microscopy. Small amount of the sample was placed in glass slide and viewed through simple microscope. Image of prepared nanoparticle shows the encapsulation of polymer mixture on drug particles.

**SCANNING ELECTRON MICROSCOPE:** [77, 78, 79.]

The surface characteristics of optimized formulation (CD10) particle size were studied by SEM. The SEM image of prepared nanoparticle formulation shows the coating of polymer mixture on drug particles. The size distribution of nanoparticles indicates a thin and uniform coating over the drug.

**SURFACE CHARGE (ZETA POTENTIAL):** [80.]

Zeta potential of glimepiride cyclodextrin complexes is commonly used to characterise the surface property of nanoparticle. It reflects the electrical potential of the nanoparticle and is influenced by the composition of the particle and the medium in which it is dispersed.

The zeta potential of the formulation showed zeta potential of -4.65mV which confirmed that the particle of the formulation remains stable.

Zeta potential result shows good and the value is increased due to the fact that the surface free energy of β- cyclodextrin is increased. The size distribution report by intensity showed the average size of 170.3 nm.

# Zeta Potential Report

*v2.3*

Malvern



## Sample Details

**Sample Name:** VM potential 1
**SOP Name:** mansettings.nano
**General Notes:**

**File Name:** ts4.dts
**Record Number:** 1024
**Date and Time:** Tuesday, November 19, 2013 4:5...
**Dispersant Name:** Water
**Dispersant RI:** 1.330
**Viscosity (cP):** 0.8872
**Dispersant Dielectric Constant:** 78.5

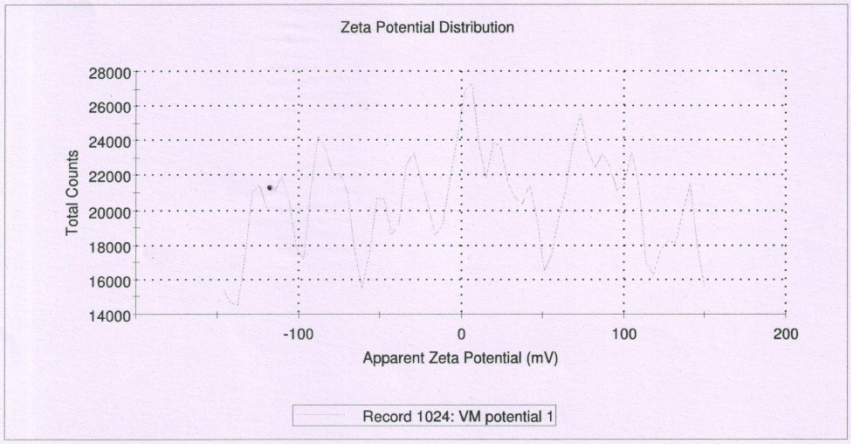
## System

**Temperature (°C):** 25.1
**Count Rate (kcps):** 7.1
**Cell Description:** Zeta dip cell
**Zeta Runs:** 26
**Measurement Position (mm):** 4.50
**Attenuator:** 11

## Results

| | | Mean (mV) | Area (%) | St Dev (mV) |
|---|---|---|---|---|
| Zeta Potential (mV): -4.65 | **Peak 1:** | -79.6 | 11.4 | 10.9 |
| **Zeta Deviation (mV):** 164 | **Peak 2:** | 0.112 | 11.3 | 9.81 |
| **Conductivity (mS/cm):** 0.792 | **Peak 3:** | 67.7 | 10.5 | 9.91 |

**Result quality :** See result quality report




Malvern Instruments Ltd
www.malvern.com

Zetasizer Ver. 7.02
Serial Number : MAL1063652

File name: ts4.dts
Record Number: 1024
19 Nov 2013 5:00:49 PM

## Size Distribution Report by Intensity

*v2.2*

Malvern

### Sample Details

**Sample Name:** VM size 1
**SOP Name:** mansettings.nano
**General Notes:**

| | | | |
|---|---|---|---|
| **File Name:** | ts4.dts | **Dispersant Name:** | Water |
| **Record Number:** | 1023 | **Dispersant RI:** | 1.330 |
| **Material RI:** | 1.59 | **Viscosity (cP):** | 0.8872 |
| **Material Absorbtion:** | 0.010 | **Measurement Date and Time:** | Tuesday, November 19, 2013... |

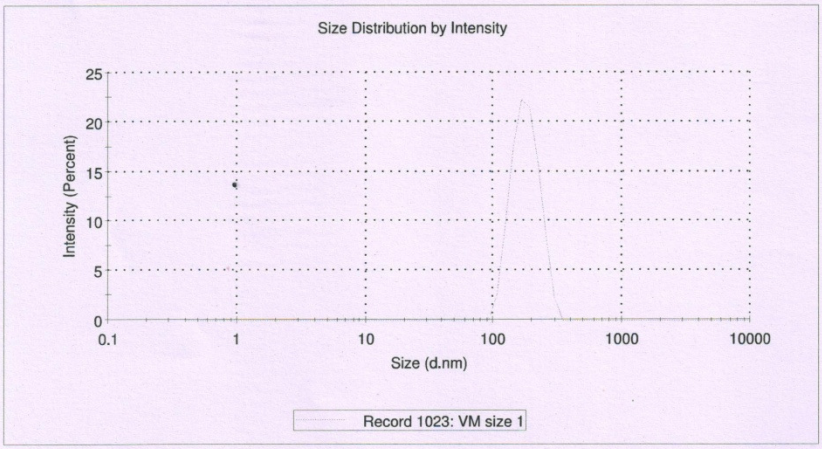
### System

| | | | |
|---|---|---|---|
| **Temperature (°C):** | 25.1 | **Duration Used (s):** | 70 |
| **Count Rate (kcps):** | 184.1 | **Measurement Position (mm):** | 4.65 |
| **Cell Description:** | Disposable sizing cuvette | **Attenuator:** | 10 |

### Results

| | | | Size (d.nm): | % Intensity: | St Dev (d.n... |
|---|---|---|---|---|---|
| Z-Average (d.nm): | 170.3 | **Peak 1:** | 179.7 | 100.0 | 42.77 |
| PdI: | 0.030 | **Peak 2:** | 0.000 | 0.0 | 0.000 |
| **Intercept:** | 0.683 | **Peak 3:** | 0.000 | 0.0 | 0.000 |

**Result quality :** Good

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Zetasizer Ver. 7.02
Serial Number : MAL1063652

File name: ts4.dts
Record Number: 1023
19 Nov 2013 4:59:34 PM

**STABILITY TESTING OF GLIMEPIRIDE NANOPARTICLES:**

The stability studies of the optimized formulation F10 was carried out for 3 months at $4^0$ C, room temperature and $45^0$ C/70% RH. At the time interval of 1 month the nanoparticle formulation was evaluated for entrapment efficiency. The stability of the formulation was stable at $4^0$C when compared to room temperature and at $45^0$C/70%RH.

**Table 21: Stability studies of nanoparticles:**

| S.No | Storage condition | Test parameters | 1st month | 2nd month | 3rd month |
|---|---|---|---|---|---|
| 1 | $4^0$ C | pH<br>Colour<br><br>*Invitro* % release at 24th hr | 7.4<br>Clear and colourless<br><br>95.11 | 7.4<br>Clear and colourless<br><br>95.18 | 7.4<br>Clear and colourless<br><br>95.20 |
| 2 | Room temperature | pH<br>Colour<br><br>*Invitro* % release at 24th hr | 7.4<br>Clear and colourless<br><br>95 | 7.4<br>Clear and colourless<br><br>90.97 | 7.4<br>Clear and colourless<br><br>90.16 |
| 3 | Acceleration condition at $45^0$C/70%RH | pH<br>Colour<br><br>*Invitro* % release at 24th hr | 7.3<br>Clear and colourless<br><br>95 | 7.3<br>Clear and colourless<br><br>81.66 | 7.3<br>Clear and colourless<br><br>80.65 |

**Table 22: In vitro release for optimized formulation CD10 stability study at $4^0$ C:**

| Time (Hrs) | Cumulative % drug release | | |
|---|---|---|---|
| | 1st month (%) | 2nd month (%) | 3rd month (%) |
| 1 | 12.65 | 12.4 | 11.01 |
| 2 | 15.12 | 15.10 | 14.96 |
| 3 | 20.17 | 19.75 | 19.54 |
| 4 | 22.78 | 22.17 | 21.09 |
| 5 | 25.22 | 25.10 | 24.85 |
| 6 | 30.27 | 29.95 | 29.56 |
| 7 | 35.32 | 35.01 | 34.12 |
| 8 | 37.85 | 36.15 | 35.97 |
| 9 | 42.55 | 42.09 | 41.68 |
| 10 | 47.57 | 46.54 | 46.09 |
| 11 | 52.54 | 51.23 | 51.02 |
| 12 | 55.49 | 54.86 | 54.58 |
| 13 | 62.58 | 62.44 | 61.96 |
| 14 | 65.18 | 64.96 | 64.34 |
| 15 | 70.08 | 69.94 | 69.54 |
| 16 | 70.08 | 69.64 | 69.54 |
| 17 | 75.18 | 74.75 | 73.65 |
| 18 | 80.18 | 79.92 | 78.25 |
| 19 | 80.18 | 79.92 | 79.43 |
| 20 | 82.54 | 81.25 | 80.24 |
| 21 | 87.58 | 87.18 | 86.18 |
| 22 | 90.19 | 89.81 | 89.51 |
| 23 | 92.51 | 91.18 | 91.10 |
| 24 | 95.11 | 95.18 | 95.20 |

**Fig 14: Stability study release data for formulation CD10 after 3 months at $4^0$C:**

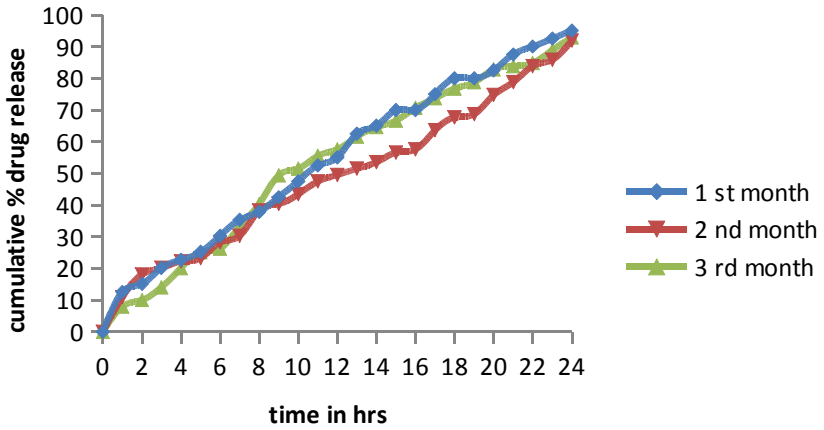


**Table 23: In vitro data for optimized formulation CD10 stability study at room temperature:**

| Time (Hrs) | Cumulative % drug release | | |
|---|---|---|---|
| | 1st month (%) | 2nd month (%) | 3rd month (%) |
| 1 | 12.68 | 01.06 | 01.04 |
| 2 | 15.12 | 08.05 | 07.05 |
| 3 | 20.17 | 11.07 | 10.95 |
| 4 | 22.7 | 16.10 | 15.60 |
| 5 | 25.22 | 17.15 | 16.45 |
| 6 | 30.27 | 19.16 | 18.56 |
| 7 | 35.32 | 26.18 | 26.08 |
| 8 | 37.85 | 29.25 | 29.17 |
| 9 | 42.54 | 34.28 | 33.85 |
| 10 | 47.59 | 35.33 | 35.53 |
| 11 | 52.58 | 40.34 | 39.44 |
| 12 | 55.08 | 42.39 | 41.59 |
| 13 | 62.58 | 46.41 | 45.31 |
| 14 | 65.08 | 48.45 | 48.25 |
| 15 | 70.45 | 56.47 | 56.17 |
| 16 | 70.45 | 58.55 | 57.55 |
| 17 | 75.18 | 62.57 | 67.97 |
| 18 | 80.71 | 67.61 | 66.81 |
| 19 | 80.71 | 70.66 | 70.15 |
| 20 | 82.54 | 73.69 | 72.59 |
| 21 | 87.51 | 75.72 | 75.18 |
| 22 | 90.74 | 77.74 | 77.54 |
| 23 | 92.58 | 83.76 | 80.76 |
| 24 | 95.05 | 90.97 | 90.16 |

**Fig 15: Stability study release data for formulation CD10 after 3 months at room temperature:**

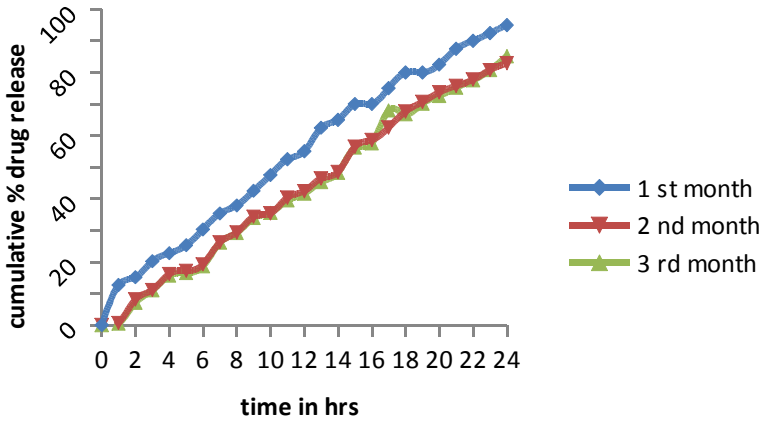
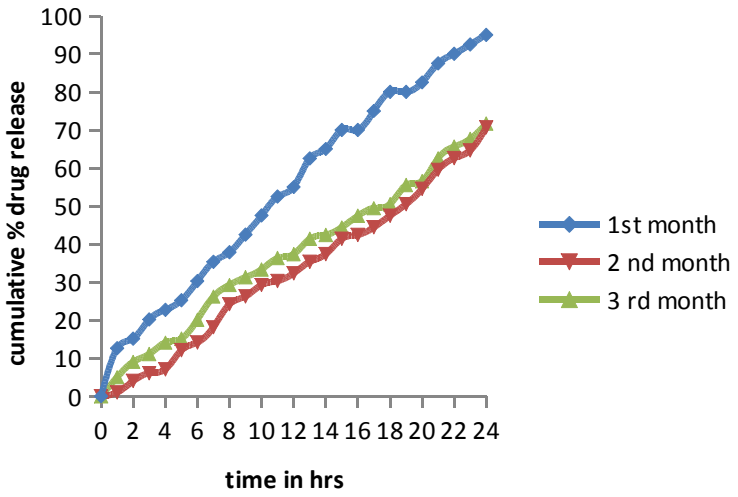


**Table 24: In vitro data for optimized formulation CD10 stability study at 45$^0$C/ 75%RH:**

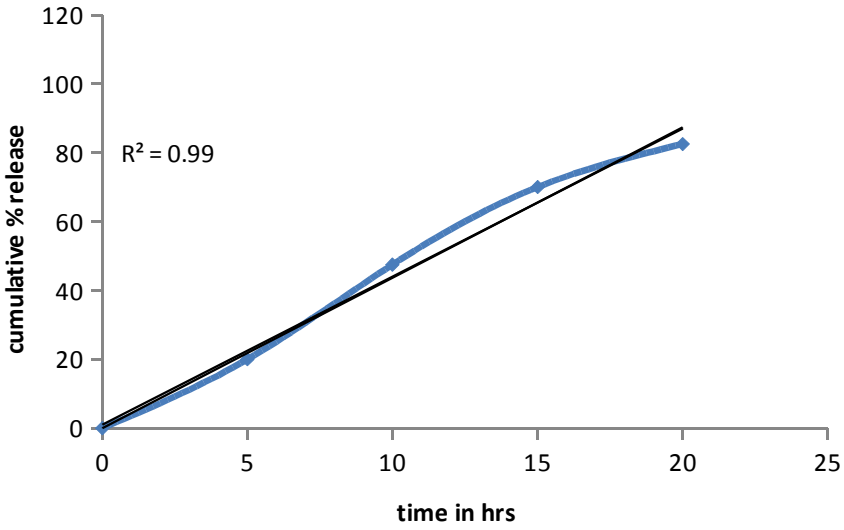
| Time (Hrs) | Cumulative % drug release | | |
|---|---|---|---|
| | 1st month (%) | 2nd month (%) | 3rd month (%) |
| 1 | 12.61 | 05.0 | 01.05 |
| 2 | 15.12 | 09.04 | 04.02 |
| 3 | 20.17 | 11.08 | 06.05 |
| 4 | 22.73 | 14.10 | 07.07 |
| 5 | 25.22 | 15.13 | 12.08 |
| 6 | 30.27 | 20.14 | 14.13 |
| 7 | 35.32 | 26.19 | 18.15 |
| 8 | 37.85 | 29.25 | 24.19 |
| 9 | 42.55 | 31.28 | 26.25 |
| 10 | 47.54 | 33.30 | 29.26 |
| 11 | 52.57 | 36.32 | 30.30 |
| 12 | 55.81 | 37.35 | 32.31 |
| 13 | 62.55 | 41.36 | 35.33 |
| 14 | 65.18 | 42.40 | 37.36 |
| 15 | 70.18 | 44.41 | 41.38 |
| 16 | 70.18 | 47.43 | 42.42 |
| 17 | 75.18 | 49.45 | 44.43 |
| 18 | 80.82 | 50.48 | 47.45 |
| 19 | 8082 | 55.49 | 50.48 |
| 20 | 82.58 | 56.54 | 54.51 |
| 21 | 87.52 | 62.55 | 59.55 |
| 22 | 90.18 | 65.61 | 62.60 |
| 23 | 92.59 | 77.64 | 74.63 |
| 24 | 95.15 | 81.66 | 80.65 |

**Fig 16: Stability study release data for formulation CD10 after 3 months at 45$^0$C/75% RH:**



**KINETICS OF DRUG RELEASE FOR OPTIMIZED FORMULATION CD10:**

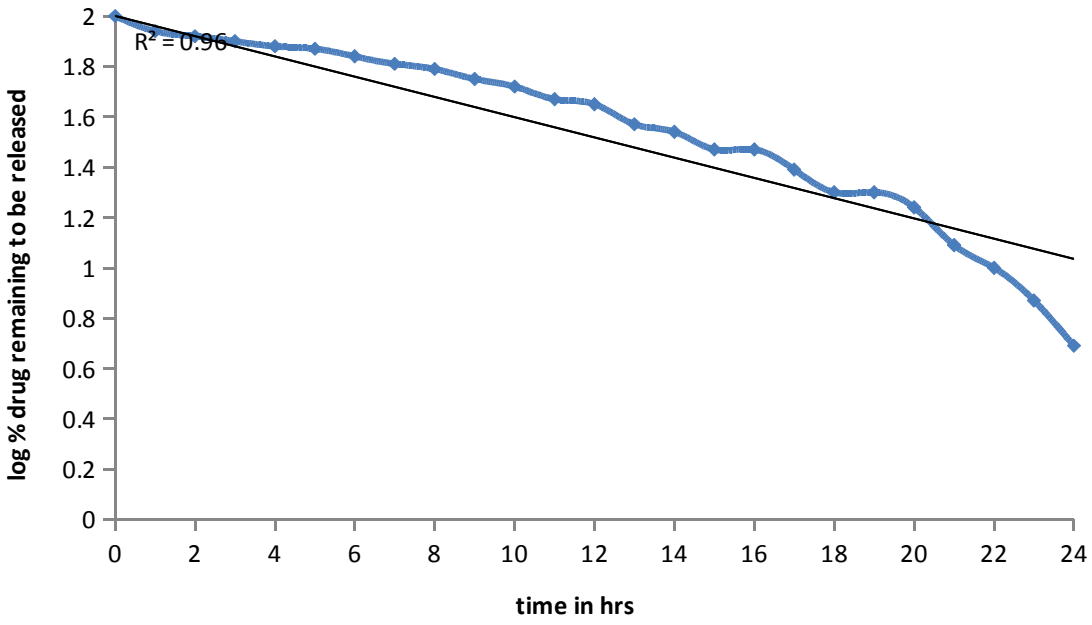
The optimized formulation CD10 was introduced into graphical treatment for kinetics of drug release.




The optimized formulation CD of nanoparticles is more suitable for parentral administration it shows good in the in vitro kinetic study. The zero order plots wee obtained by plotting cumulative percentage drug release versus time. The regression value is 0.987.

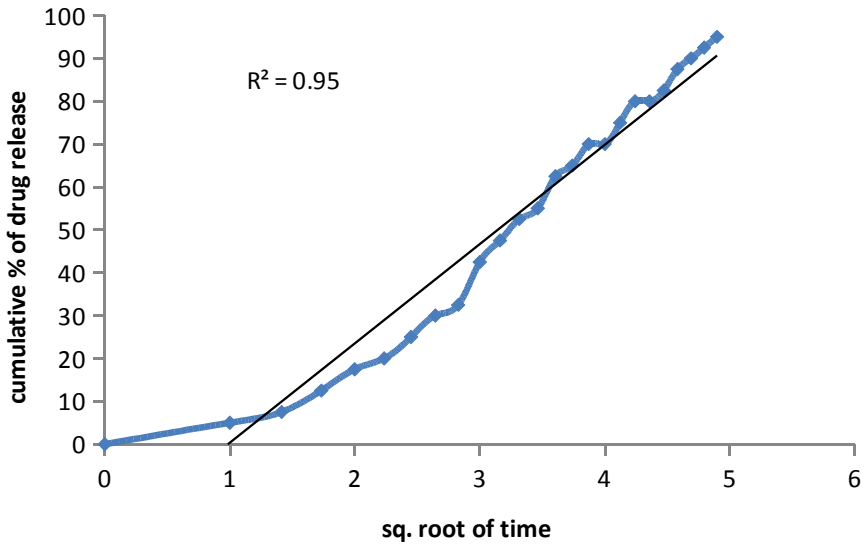
**FIRST ORDER KINETICS OF DRUG RELEASE:**

The first order plot was made by plotting log time remaining cumulative % drug release against time. The regression value was found to be which indicates that drug release does not follow first order rate kinetics.



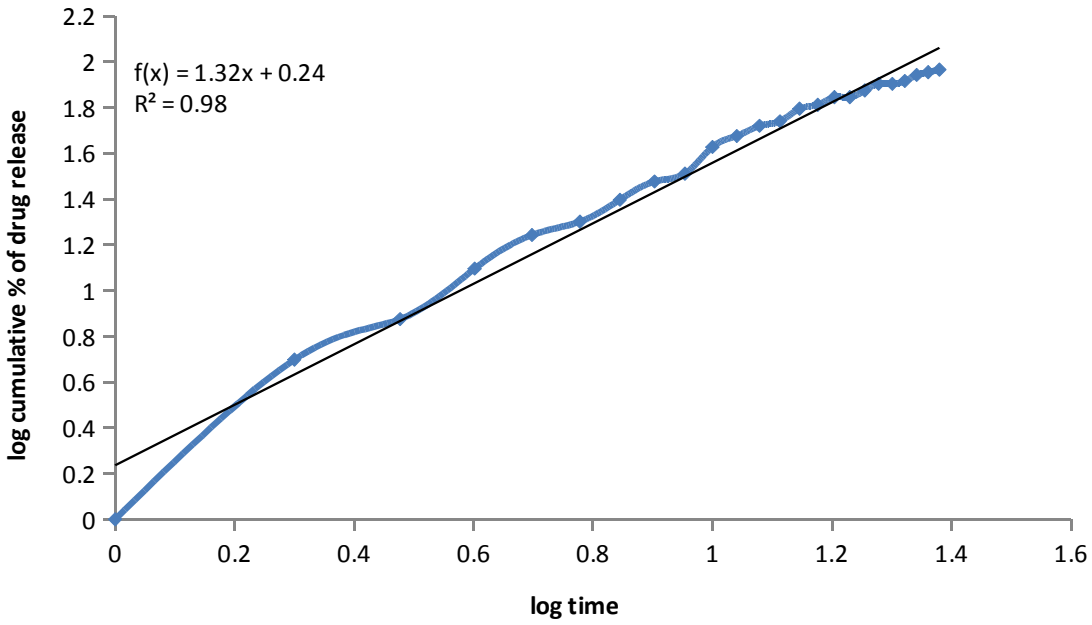
**HIGUCH'S PLOT:**

Higuchi plot was made by plotting cumulative % drug release against square root of time. The regression value was found to be 0.951. This indicates that diffusion is one of the mechanisms of drug release.



**KORSEMEYER PLOT:**

The graph was plotted between log cumulative % of drug release and log time. [the value was found to be 0.992 anomalous (non – Fickian) diffusion.



## SUMMARY AND CONCLUSION

The present study glimepiride nanoparticles aimed to develop a nanoparticulate drug delivery system using cyclodextrin inclusion complexes.

The polymer enhances the binding of glimepiride nanoparticles in specific or targeted site with sustained release of drug which increases the therapeutic efficacy. These nanoparticles may also reduce the dose and dose frequency with desired therapeutic response.

The preformulation studies were performed using FTIR. The spectra pure drug and the formulation were examined. The study revealed the absence of interaction between drug and the polymer.

All the batches of nanoparticles (CD1-CD10) were prepared by kneading method. Formulation was subjected to following evaluation tests which involves;

- Entrapment efficiency
- In vitro drug release studies
- Microscopic determination
- Particle size determination

The entrapment efficiency of the optimized formulation was 94±0.05% and the in vitro drug release was 94.89% after 24 hrs. It obeys zero order, follows diffusion and erosion mechanism of release. Particle size determination by SEM shows the best formulation containing size of 170 nm. Therefore it can target the tissues and has a quick onset of action. The optimized formulation was examined for zeta potential determination. The formulation CD10 showed maximum deviation of -4.65 mV which demonstrates that the particles are separate and highly repelling. The repelling property was more useful in decreasing opsonisation. Further studies are to be carried out to reduce the side effects.

Therefore, formulated glimepiride nanoparticles can be expected to gain considerable attention in the treatment of type 2 diabetes mellitus due to its improved therapeutic activity.

1. Rainer H. Muller, Karsten Mader and Sven Gohla, Solid lipid nanoparticles (SLN) for controlled drug delivery, review of the state of art, *European Journal of Pharmaceutics and Biopharmaceutics;* 2000; 50(1): 161-177.
2. Targeted delivery of drugs, Controlled and novel drug delivery by N.K. Jain, (1), 93.
3. Targeted delivery of drugs, Advances in controlled and Novel drug delivery by N.K Jain; (1): p.452.
4. J. Adlin Jino Nesalin, A. Anton Smith, Nanoparticles an invisible drug delivery system, Review article, *Journal of Pharmacy Research*; 2011; 4: p. 373-377.
5. Polymers in controlled release, controlled drug delivery by Joseph R. Robinson; (2): p.179.
6. Oral controlled release drug delivery system, controlled drug delivery by Joseph R. Robinson; (2), p. 385.
7. Hormones and hormone antagonists, the pharmacological basis of therapeutics; (11), p. 1613.
8. Pancreatic hormones, anti diabetic drugs, Pharmacology and pharmacotherapeutics, R.S. Satoskar, 2013; (23): p.906.
9. Insulin, oral hypoglycaemic drugs and glucagon, essentials of medical pharmacology, K.D. Tripathi, p.258.
10. The endocrine pancreas and the control of blood glucose, Rang and Dale's pharmacology; (6), p. 402.
11. Pedro Tartaj, Marıadel Puerto Morales, Sabino Veintemillas-Verdaguer, Teresita Gonzalez-Carreno and Carlos J Serna, The preparation of magnetic nanoparticles for applications in biomedicine, *journal of physics and applied physics*; 2003; 36(3): 182-197.
12. Mansoor Amiji and Sushma Kommareddy, Preparation and Evaluation of Thiol-Modified Gelatin Nanoparticles for Intracellular DNA Delivery in Response to Glutathione, Bioconjugate Chem; 2005; 16(6): 1423-1432.
13. Kunihiko Koshiba, Masahiro Nomura, Yutaka Nakaya and Susumu Ito, Efficacy of glimepiride on insulinresistance, adipocytokines, and atherosclerosis, The Journal of Medical Investigation; 2006; 53(1):87-94.
14. Catarina Pinto Reis, Ronald J. Neufeld, Antonio J. Ribeiro, Francisco Veiga, nanoencapsulation methods for preparation of drug-loaded polymeric nanoparticles, nanomedicine: nanotechnology, biology and medicine; 2006; 2(1): 8-21.
15. H.O.Ammar, H.A.Salama, M. Ghorab, A.A.Mahmoud, Inclusion complexation of glimepiride in dimethyl-β-cyclodextrin, *Asian Journal of Pharmaceutical Sciences;* 2007; 2 (2): 44-55.
16. Manivannan Rangasamy, Ayyasamy Balasubramaniam and Sandeep Gummadevelly, Design and Evaluation of β-cyclodextrin Complexes of Meloxicam Tablet, Research J. Pharm. and Tech; 2008; 1(4);484-486.

17. V. G. Kuchake, R. D. Shimpi, P. H. Patil, P.V.Ingle, S. J. Surana, P. N. Dighore , Comparison of effect of metformin in combination with glimepiride and glibenclamide on glycaemic control in patient with type 2 diabetes mellitus, International Journal of Pharm Tech Research; 2009; 1 (1): 50-61.
18. S. Tamizhrasi, A. Shukla, T. Shivkumar, V. Rathi , J. C. Rathi,formulation and evaluation of lamivudine loaded polymethacrylic acid nanoparticles, *International Journal of PharmTech Research* CODEN( USA): IJPRIF; 2009; 1 (3); 411-415.
19. Vipul P. Patel, Natvarlal M. Patel, Evaluation of Some Methods for Preparing Glipizide-β β- Cyclodextrin Inclusion Complexes, Iranian Journal of Pharmaceutical Sciences Autumn; 2009; 5(4): 191-198.
20. N.Arunkumar, M.Deecaraman, C.Rani, K.P.Mohanraj, K.Venkates Kumar, Preparation and solid state characterization of atrovastatin nanosuspension for enhanced solubility and dissolution, International Journal of PharmTech Research; 2009; 1(4); 1725-1730.
21. L. Zhang, D. Pornpattananangkul, C.-M.J. Hu and C.-M. Huang, Development of Nanoparticles for Antimicrobial Drug Delivery, *Current Medicinal Chemistry*; 2010; 17(6); 585-594.
22. Carmelia Nicolescu, Ciruna Arama, Angela Nedelcu, Crina- Maria Monciu, phase solubility studies of the inclusion complexes of repaglinide with β- cyclodextrin and β- cyclodextrin derivatives, Farmacia;2010; 58(5);620-628.
23. Rezaei Mokarram, Kebriaee zadeh, Keshavarz M, Ahmadi , Mohtat, Preparation and in-vitro evaluation of indomethacin nanoparticles, DARU; 2010; 18 (3);185-192.
24. Mitra Jelvehgari, Jaleh Barar, Hadi Valizadeh, Nasrin Heidari, Preparation and Evaluation of Poly (ε-caprolactone) Nanoparticles-in- Microparticles by W/O/W Emulsion Method, Iranian Journal of Basic Medical Sciences; 2010; 13(3): 85-96.
25. Partha Saha, Amit K Goyal and Goutam Rath, Formulation and Evaluation of Chitosan-Based Ampicillin Trihydrate Nanoparticles, *Tropical Journal of Pharmaceutical Research;* 2010; 9 (5): 483-488.
26. S. Debnath, D. Datta, M.N. Babu, R.S. Kumar,V.Senthil, studies in preparation and evaluation of chitosan nanoparticles containing cytarabine, international journal of pharmaceutical secinces and nanotechnology; 2010; 3(2):957-964.
27. C. Rubina Reichal, J. Bhagya Lakshmi and T. K Ravi, Studies on formulation and in vitro evaluation of Glimepiride floating tablets, *Journal of Chemical and Pharmaceutical Research*; 2011; 3(3):159-164.
28. Narasimha Reddy D, Srinath MS , Hindustan Abdul Ahad , Kishore Kumar Reddy B, Vamsi Krishna Reddy P , Krishna Mahesh Ch , Kranthi G , Raghavendra P,

Formulation and in-vitro Evaluation of Glimepiride and Parecoxib Combination Mucoadhesive Tablets, *Scholars Research Library*; 2011; 3(1):185-192.

29. Neha Chowdhary, Meenakshi Bajpai, formulation, characterisation and invitro evaluation of cyclodextrin complexed oro dispersible tablets of glimepiride, international journal of research in pharmaceutical sciences; 2011; 2(4): 512-521.
30. Veerendra S. Rajpurohit, Pankaj Rakha, Surender Goyal, Harish Dureja, Gitika Arora and Manju Nagpal, Formulation and Characterization of Solid Dispersions of Glimepiride through Factorial Design, *Iranian Journal of Pharmaceutical Sciences Winter;* 2011; 7(1): 7-16.
31. Shahrooz Saremi, Fatemeh Atyabi1, Seyedeh Parinaz Akhlaghi, Seyed Nasser Ostad, Rassoul Dinarvand, Thiolated chitosan nanoparticles for enhancing oral absorption of docetaxel: preparation, in vitro and ex vivo evaluation, International Journal of Nanomedicine; 2011:6 ;119–128.
32. Durga Prasad Pattanayak and Subash C. Dinda, Bilayer tablet formulation of metformin hydrochloride and glimepiride: A novel approach to improve therapeutic efficacy, *international journal of drug discovery and herbal;* 2011; 1(1): 1-4.
33. Adel M. Aly, Bassam I. Amro and Feras D. El Hajji, Preparation and Evaluation of Rapidly Disintegrating Glimepiride Tablets, International Journal of Pharmaceutical Sciences and Nanotechnology; 2011; 3(4);1220-1229.
34. Anilkumar J. Shinde, Harinath N. More, design and evaluation of polylactic-co-glycolic acid nanoparticles containing simvastatin, International Journal of Drug Development & Research; 2011; 3(2); 280-289.
35. Subhranshu Panda, Sheetal acharya, Jyoti Gandhi, Nishith Patel, Development and Characterization of Mucoadhesive patches of Glimepiride for buccal administration, *journal of pharmaceutical science and bio scientific research;* 2011;1(2); 102-107.
36. Chandra Sekhar Y, V.Venu, K.Jaganathan, R.Senthil selvi, P.Perumal,formulation and invitro evaluation of sustained release matrix tablets of glimepiride by using natural gums as release modifiers, *Journal of Global Trends in Pharmaceutical Sciences;* 2011; 2(4): 394-403.
37. Khushbu Shenghani, Yogesh Chaudhari, Suresh Kumavat, Priyanka Borole, Pankit ShahKhushbu Shenghani, Yogesh Chaudhari, Suresh Kumavat, Priyanka Borole, Pankit Shah Patel Naveen, L Aparna, S Uma, Patil Swaraj, Design and Characterisation of Mucoadhesive Buccal Patch of Glimepiride, *International Journal of Research in Pharmacy and Science*; 2012; 2(1): 117-128.
38. Mohammed S. Khan , Rohitash K , Vijaykumar M , Suresh C Pandey , Gowda D. Vishakante , Faruqui M. Ahmed , Aquil R Sidiqui , Development and evaluation of

nasal mucoadhesive nanoparticles of an analgesic drug, *Der Pharmacia Lettre*; 2012; 4 (6): 1846-1854.

39. Lakshmi Sirisha Kotikalapudi, Laxminarayana Adepu, J. VijayaRatna, Prakash V Diwan, Formulation and invitro characterization of domperidone loaded solid lipid nanoparticles, *Int J Pharm Biomed Res;* 2012; 3(1): 22-29.
40. Ghanshyam Patel, Dr.Rajin Shah, Bhavin Bhimani,Rajendra Patel, Dr.Upendra patel,Dhiren Daslaniya,Formulation and evaluation of bilayered tablet of Metformin hydrochloride and glimepiride, *international journal of pharmaceutical research and bio-science*; 2012; 1(6): 75-87.
41. Naik J B, Mokale V J, Formulation and evaluation of Repaglinide nanoparticles as sustained release carriers, *Novel Science International Journal of Pharmaceutical Science;* 2012; 1(5): 259-266.
42. Madhusudhanareddy Induri, Bhagavan Raju M, Rajendra Prasad Y and Pavankumar Reddy, Development and Validation of A Spectrophotometric Method for Quantification and Dissolution Studies of Glimepiride in Tablets, *E-Journal of Chemistry;* 2012; 9(2): 993-998.
43. Mohd Abdul Hadi, V. Lokeswara Babu and Narottam Pal, Formulation and Evaluation of Sustained Release Matrix Tablets of Glimepiride Based on Combination of Hydrophilic and Hydrophobic Polymers, *Journal of Applied Pharmaceutical Science;* 2012; 2 (6): 101-107.
44. Wagh V. T., Jagtap V.A., Shaikh T.J., Nandedkar S.Y., Formulation and Evaluation of Glimepiride Solid Dispersion Tablets for Their Solubility Enhancement, *Journal of Advanced Scientific Research*; 2001; 3(4): 36-41.
45. Neha Pachisia and Shyam Sunder Agrawal, Formulation, development and evaluation of transdermal drug delivery system of Glimepiride, International Journal of Pharmacy and Pharmaceutical Science Research; 2012; 2(1): 1-8.
46. Hari Har Prasad.M, Duraivel.S, Effect of Different Binders and Super Disintegrants on Formulation of Glimepiride Immediate Release Tablets by Wet Granulation Method, *International Journal of Pharmaceutical and Clinical Research;* 2012; 4(4): 44-47.
47. Patel Naveen, L Aparna, S Uma, Patil Swaraj, Design and Characterisation of Mucoadhesive Buccal Patch of Glimepride, International Journal of Research in Pharmacy and Science; 2012; 2(1):117-128.
48. Sreenivasa Rao K, Mohammed Majid Iqbal and Prabhakar Shirse, Preparation and Evaluation of Cyclodextrin Inclusion Complexes of Water Insoluble Drug-Glimipiride, International Journal of Research in Pharmaceutical and Biomedical Sciences; 2012; 3 (1): 2229-2237.

49. P. Srinivas S. Pragna, Formulation and evaluation of Moxifloxacin hydrochloride ocular nanoparticle, *International Journal of Nano Dimension;* 2012; 3 (2): 105-113.
50. Jaimin D patel, Sachin P Chauhan, A. K. Seth, formulation and evaluation of methotrexate loaded nanoparticle, *International Journal of Drug Discovery and Medical Research*; 2012; 1(2);56-60.
51. Shantanu Tamuly and Mumtesh Kumar Saxena, Preparation of calcium phosphate nanoparticles and evaluation of their effects on muscle cells of rat, *current science*; 2012; 102(4):610-612.
52. Swati Srivastava, GN Saxena, P Keshwani, Ritesh Gupta, Comparing the Efficacy and Safety Profile of Sitagliptin versus Glimepiride in Patients of Type 2 Diabetes Mellitus Inadequately Controlled with Metformin Alone, Journal of the association of physicians of India; 2012; (60): 27-30.
53. Hindustan Abdul Ahad, Sreeramulu J,Narasimha Reddy D, Sravanthi M and Suma Padmaja B, preparation of glimepiride sustained release matrix tablets using Hibiscus rosa-sinensis leaves mucilage and Povidone, Indian journal of pharmaceutical education and research; 2012; 46(2): 770-777.
54. Shirse Prabhakar, formulation and evaluation of fast dissolving tablets of cyclodextrin inclusion complexed water insoluble drug: glimepiride, IJRAP 2012; 3(3): 465-470.
55. Prabhakar Shirse, formulation and evaluation of bilayered tablets of HP-β-cyclodextrin complexed glimepiride with metformin hydrochloride for immediate and sustain release, *International Journal of Pharma and Bio Sciences*; 2012; 3(3): 148 – 160.
56. Kishore K, Sudhakara Reddy P, Srininvas Reddy D, Maheshwar T, Kiran Kumar V, Raju L, Preparation and characterization of oro dispersible tablets of glimepiride-PVP K30 solid dispersion, *International Journal of Biological & Pharmaceutical Research*; 2013; 4(8): 547-555.
57. M. Tejakrishna, V.Sai Kishore, K.V.S.Prasada Rao, B.Sudheer, Formulation and Evaluation of Mucoadhesive Microbeads of Glimepiride*, Scholars Academic Journal of Pharmacy;* 2013; 2(3):199-208.
58. Ram Alpana, development and evaluation of solid dispersions of tolbutamide, *Asian Journal of Pharmaceutical and Clinical Research*; 2013; 6(1):220-224.
59. Yashavantsinh Chavd, Bhavin Bhimani, Upendra Patel, Ragin Shah, Ghanshyam Patel, Dhiren Daslaniya, preparation and avaluation of lamivudine microspheres with Eudragit polymers by solvent evaporation method for lymphatic system, international journal of pharmaceutical research and bio- science; 2013; 2(3): 89-106.

60. Vyjayanthimala T, Bharathi, Lakshmi Radhika G, Mallamma T, Dinesh Rawal, formulation and evaluation of Zidovudine loaded chitosan nanoparticles for antiviral therapy, *International Journal of Biopharmaceutics*; 2014; 5(1): 1-4.
61. Yun Mo, Lee-Yong Lim, preparation and invitro anticancer activity of wheat germ agglutinin (WGA)- conjugated PLGA nanoparticles loaded with paclitaxel and isopropyl myristate, journal of controlled release; 2005; 107(1): 30-42.
62. L.X. Tiefenauer, A. Tschirky, G. Iwhne and R.Y. Andres, In vivo evaluation of magnetite nanoparticles for use as a tumor contrast agent in MRI, Magnetic Resonance Imaging; 1996; 14(4): 391-402.
63. Asuman Bozkir, Ongun Mehmet Saka, Formulation and investigation of 5-FU nanoparticles with factorial design-based studies, farmac; 2005; 60(10): 840-84.
64. C. Schwarz, W. Mehnert, Freeze-drying of drug-free and drug-loaded solid lipid nanoparticles (SLN), International Journal of Pharmaceutics; 1997; 157(2): 171-179.
65. P.K. Gupta and C.T. Hung, Quantitative evaluation of targeted drug delivery systems, Elsevier Science Publishers B.V. (Biomedical Division); 1989; 56(3): 217-226.
66. Adlin Jino Nesalin J, Gowthamarajan K and Somashekharac, Formulation and evaluation of nanoparticles containing Flutamide, *International Journal of Chemistry Tech Research*; 2009; 4(5): 1331-1334.
67. S. Tamizhrasi, A. Shukla, V.Rathi, J.C. Rathi, Formulation and evaluation of Lamivudine loaded polymethacrylic acid nanoparticles, *International Journal of Pharm Tech Research;* 2009; 1(3): 411-415.
68. Le Thi Hoa, Nguyen Tai Chi, Nguyen Minh Triet, Le Ngoc Thanh Nhanand, Dang Mau Chien, Preparation of drug nanoparticles by emulsion evaporation method, *Journal of Pharmaceutics;* 2009; 187(1): 012-047.
69. Ahmed H. Elshafeey, Amany, Kamel, Gehanne, Ammonium methacrylate unit polymer content effect on actclovir colloidal nanoparticles properties and bioavailability in human volunteers, *Journal of colloidal and surfaces B: Biointerfaces*; 2010; 7(6): 398-404.
70. Rubina Mara Mainardesa, Najeh Maissar Khalil, Maia Palmira Daflon Gremiaob, Intranasal delivery of Zidovudine by PLA and PLA-PEG blend nanoparticles, *International Journal of Pharmaceutics;* 2010; 39(3): 266-271.
71. Selvakumar Kalimuthu, A. V. Yadav, formulation and evaluation of carvedilol loaded Eudragit e 100 nanoparticles, *International Journal of Pharm Tech Research*; 2009;1( 2): 179-125.
72. Ahmed H. Elshafeey, Amany, Kamel, Gehanne, Ammonium methacrylate unit polymer content effect on actclovir colloidal nanoparticles properties and

bioavailability in human volunteers, *Journal of colloidal and surfaces B: Biointerfaces*; 2010; 75(2): 398-404.

73. Angela Loopedota, Adriana Trapani, Annalisa Cutrignelli, Laura Chiarantini, Elena Pantucci, Rosa Cucri, Elisabetta Manuali, Giuseppe Trapani, The use of Eudragit RS 100/ cyclodextrin nanoparticles for the transmucosal administration of glutathione, *European Journal of Pharmaceutics and Biopharmaceutics*; 2009; 72(3): 509-520.
74. Roberta Caravalli, Otto Caputo, Maria Eugenia Carlotti, Michele Trotta, Carmela Scarnecchia, Maria Rosa Gasco, Sterilization and freeze-drying of drug-free and drug-loaded solid lipid nanoparticles, International Journal of Pharmaceutics; 1997; 148(1): 47-54.
75. Partha Saha, Amit K Goyal and Goutam Rath, Formulation and Evaluation of Chitosan-Based Ampicillin Trihydrate Nanoparticles, *Tropical Journal of Pharmaceutical Research;* 2010; 9 (5): 483-488.
76. R. Pignatello, C. Bucolo, G. Spedalieri, A. Maltese, G. Puglisi, Flurbiprofen loaded acrylate polymer nanosuspensions for ophthalmic application, *Journal of Biomaterials*; 2002; 23(16): 3247-3255.
77. Junping Wang, Si- Shen Feng, Shu Wang, Zhi- ying Chen, Evaluation of cationic nanoparticles of biodegradable copolymers as RNA delivery system for hepatitis B treatment*, International Journal of Pharmaceutics*; 2010; 400(2): 194-200.
78. Ugo Bilati, Eric Allemann, Eric Doelker, Development of a nanoprecipitation method intended for the entrapment of hydrophilic drug into nanoparticles, European Journal of Pharmaceutics and Biopharmaceutics; 2005; 24(5): 67-75.
79. Rosario Pignatello, Claudio Bucolo, Piera Ferrara, Adriana Maltese, Antonina Puleo, Giovanni Puglisi, Eudragit RS100 nanosuspensions for the ophthalmic controlled delivery if ibuprofen, *European Journal of Pharmaceutical sciences*; 2002(1); 16: 53-61.
80. Rubina Mara Mainardes, Najeh Maissar Khalil, Maia Palmira Daflon Gremiao, Intranasal delivery of Zidovudine by PLA and PLA-PEG blend nanoparticles, *International Journal of Pharmaceutics;* 2010; 395(2): 266-271.